Docket No.: PF-0629 USN
USSN: 09/856,679
Ref. No. 1 of 5

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07K 14/00

A2

(11) International Publication Number: **WO 00/24768**

(43) International Publication Date: 4 May 2000 (04.05.00)

(21) International Application Number: PCT/US99/24826

(22) International Filing Date: 22 October 1999 (22.10.99)

(30) Priority Data:

60/105,507	23 October 1998 (23.10.98)	US
60/108,685	16 November 1998 (16.11.98)	US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: GENES INTEGRATING SIGNAL TRANSDUCTION PATHWAYS

(57) Abstract

The present invention describes the identification, isolation, sequencing and characterization of two human CalDAG–GEF, and two human cAMP–GEF genes, which are associated with the Ras pathway. Also identified are CalDAG–GEF gene homologues in mice and cAMP–GEF gene homologues in rats. Nucleic acids and proteins comprising or derived from the CalDAG–GEFs and/or cAMP–GEFs are useful in screening and diagnosing certain Ras–associated cancers, in identifying and developing therapeutics for treatment of certain Ras–associated cancers, and in producing cell lines and transgenic animals useful as models of Ras–associated cancers.

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GENES INTEGRATING SIGNAL TRANSDUCTION PATHWAYS

Related Applications

This application claims the benefit of U.S. Application Nos. 60/105,507, filed on October 23,1998, and 60/108,685, filed on November 16, 1998.

Field of the Invention

The present invention relates generally to novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic, and research utilities for these polynucleotides and proteins.

Background of the Invention

Ras proteins are key regulators of growth, differentiation and malignant transformation. In addition, these proteins are implicated in synaptic function and region-specific learning and memory functions in the brain.

As shown schematically in Figure 1, Ras proteins cycle between inactive GDP-complexed and active GTP-complexed states. GTPase-activating proteins (GAPs) inactivate Ras proteins by stimulating hydrolysis of the bound GTP to GDP, whereas guanine nucleotide exchange factors (GEFs) activate Ras proteins by stimulating release of GDP and the uptake of GTP. So essential are GEFs to Ras action, that genetic loss of GEF function has similar effects to those induced by loss of the Ras proteins themselves. Loss of GEF function can be circumvented by mutations that constitutively activate the Ras proteins, such as an oncogene mutation, or, in some cases, through loss of GAP activity. Activated Ras proteins, which are localized at the plasma membrane, transmit signals from tyrosine kinases to a cascade of serine/threonine kinases, which delivers the signals to the cell nucleus.

Activation of Ras can result in the activation of the mitogen-activated protein (MAP) kinase (also known as extracellular-signal regulated kinase, or ERK) pathway. For example, a receptor tyrosine kinase is activated by a peptide mitogen such as epidermal growth factor (EGF). The EGF-stimulated receptor undergoes autophosphorylation of specific tyrosine residues in its cytoplasmic domain which creates phosphotyrosyl binding sites for the Src homology 2 (SH2) and/or phosphotyrosyl binding (PTB) domains of certain adapter proteins. The adapter protein becomes autophosphorylated on association with activated receptor tyrosine kinases. The GEF is stably associated with the adapter protein which, upon autophosphorylation, mediates translocation of the GEF to the plasma membrane. The GEF then activates the Ras protein. Activated Ras relays its signal downstream through a cascade of cytoplasmic proteins, including

Raf-1 serine/threonine kinase. The Ras:Raf association promotes translocation of the normally cytoplasmic Raf protein to the plasma membrane, where subsequent events lead to the activation of its kinase function. Upon activation, Raf phosphorylates and activates two MAP kinases (also known as MEKs). MEKs directly associate with the catalytic domain of Raf-1 and are phosphorylated by Raf. Activated MEKs function as dual-specificity kinases and phosphorylate tandem threonine and tyrosine residues in the MAP kinases to activate them. Once activated, the MAP kinases translocate to the nucleus where they phosphorylate and activate a variety of substrates.

Rap proteins, members of the Ras small GTPase superfamily, can inhibit Ras signaling of the Ras/Raf-1(a serine/threonine kinase)/MAP kinase pathway or, through B-Raf, can activate MAP kinase. Rap1 consists of two isoforms, Rap1A and Rap1B, which differ mainly at the C-terminus. Characteristic features of Rap1 are its geranylgeranyl modification at the C-terminus, which is responsible for membrane attachments, and a threonine residue at position 61. In most other GTPases, the corresponding residue is a glutamine. Rap proteins, like Ras proteins, cycle between inactive GDP-complexed and active GTP-complexed states. GEFs are required to activate Rap proteins by stimulating the release of GDP and the uptake of GTP.

Constitutive activation of the Ras pathway contributes to malignant transformation. In fact, the Ras gene has been implicated in many human cancers, including lung cancer, breast cancer, colorectal cancer, exocrine pancreatic cancer, and myeloid leukemia. Biological and biochemical studies of Ras action indicate that Ras functions like a G-regulatory protein since Ras must be localized in the plasma membrane and must bind with GTP in order to transform cells. Gibbs et al., 53 MICROBIOL. REV. 171-286 (1989).

Targeting components of the Ras signaling pathways has been proposed as one approach for the development of anti-Ras drugs for cancer treatment. One potential approach for targeting Ras for cancer treatment involves the use of farnesyltransferase inhibitors (FTIs). Inhibition of farnesyl-protein transferase, and thereby of farnesylation of the Ras protein, blocks the ability of Ras to transform normal cells to cancer cells. Certain inhibitors of Ras farnesylation cause an increase in soluble Ras which can act as a dominant negative inhibitor of Ras function. While soluble Ras in cancer cells can become a dominant negative inhibitor, soluble Ras in normal cells would not be an inhibitor. A cytosol-localized and activated form of Ras acts as a dominant negative Ras inhibitor of membrane-bound Ras function. Gibbs et al., 86 PROC. NAT'L ACAD.

Sci. USA 6630-34 (1989). FTIs block Ras function by preventing its post-translational modification by the farnesyl isoprenoid.

Intervention of Ras signaling at multiple or various points can significantly impact the ability of Ras to cause cellular transformation. Since Ras protein function is believed to be crucial to so many cellular processes, targeting only a subset of Ras functions by downstream intervention may provide significant advantages. Thus, there remains a need for identifying additional means for disrupting the Ras pathway. Applicants have discovered four new targets, namely GEFs specific for Rap1A , for disrupting the Ras pathway.

Summary of the Invention

Applicants have discovered four mammalian genes which have been designated CalDAG-GEFI, CalDAG-GEFII, cAMP-GEFI, and cAMP-GEFII, which encode proteins having a substrate specificity for Rap1A. The proteins encoded by CalDAG-GEFI and CalDAG-GEFII, referred to herein generally as "CalDAG-GEF," have dual binding domains for calcium and diacylglycerol. The proteins cAMP-GEFI and cAMP-GEFII, referred to herein generally as "cAMP-GEF," have a binding domain for cyclic adenosine 3', 5'-monophosphate. The present disclosure provides polypeptide and polynucleotide sequences for Mus musculus CalDAG-GEFI, Homo sapiens CalDAG-GEFI, Rattus norvegicus CalDAG-GEFII, Homo sapiens CalDAG-GEFII, Rattus norvegicus cAMP-GEFI, Homo sapiens cAMP-GEFI, Homo sapiens alternatively spliced cAMP-GEFI, Rattus norvegicus cAMP-GEFII, and Homo sapiens cAMP-GEFII. See Kawasaki et al., 95 Proc. Natl. Acad. Sci. USA 13278-83 (1998), and Kawasaki et al., 282 Sci. 2275-79 (1998), the disclosures of both of which are incorporated by reference herein.

Thus, in one series of embodiments, the present invention provides isolated nucleic acids including nucleotide sequences comprising or derived from CalDAG-GEF or cAMP-GEF, or encoding polypeptides comprising or derived from CalDAG-GEF or cAMP-GEF proteins. The sequences of the invention include the specifically disclosed sequences, splice variants of these sequences, allelic variants of these sequences, synonymous sequences, and homologous or orthologous variants of these sequences. Thus, for example, the invention provides nucleic acid sequences from the Mus musculus CalDAG-GEFI, Homo sapiens CalDAG-GEFI, Rattus norvegicus CalDAG-GEFII, Homo sapiens CalDAG-GEFII, Rattus norvegicus cAMP-GEFI, Homo sapiens cAMP-GEFI, Homo sapiens alternatively spliced cAMP-GEFI, Rattus norvegicus cAMP-GEFII, and Homo sapiens cAMP-GEFII. The present invention also provides allelic variants and homologous or orthologous sequences by providing methods by which such variants

may be routinely obtained. Because the nucleic acids of the invention may be used in a variety of diagnostic, therapeutic and recombinant applications, various subsets of the CalDAG-GEF and cAMP-GEF sequences are also provided. For example, for use in allele specific hybridization screening or PCR amplification techniques, subsets of the CalDAG-GEF and cAMP-GEF sequences, including both sense and antisense sequences, and both normal and mutant sequences, as well as intronic, exonic and untranslated sequences, are provided. Such sequences may comprise a small number of consecutive nucleotides from the sequences which are disclosed or otherwise enabled herein, but preferably include at least 8-10, more preferably 10-15, and most preferably 15-25, consecutive nucleotides from a CalDAG-GEF or cAMP-GEF sequence. In another embodiment, such sequences include at least 25-500 consecutive nucleotides from CalDAG-GEF or cAMP-GEF sequence. Other preferred subsets of a CalDAG-GEF or cAMP-GEF sequence include those encoding one or more of the functional domains or antigenic determinants of the CalDAG-GEF or cAMP-GEF protein and, in particular, may include either normal (wild-type) or mutant sequences. The invention also provides for various nucleic acid constructs in which CalDAG-GEF or cAMP-GEF sequences, either complete or subsets, are operably joined to exogenous sequences to form cloning vectors, expression vectors, fusion vectors, transgenic constructs, and the like. Thus, in accordance with another aspect of the invention, a recombinant vector for transforming a mammalian or invertebrate tissue cell to express a normal or mutant CalDAG-GEF and/or cAMP-GEF sequence in the cells is provided.

In another series of embodiments, the present invention provides for host cells which have been transfected or otherwise transformed with one of the nucleic acids of the invention. The cells may be transformed merely for purposes of propagating the nucleic acid constructs of the invention, or may be transformed so as to express the CalDAG-GEF and/or cAMP-GEF sequences. The transformed cells of the invention may be used in assays to identify proteins and/or other compounds which affect normal or mutant CalDAG-GEF and/or cAMP-GEF expression, which interact with the normal or mutant CalDAG-GEF and/or cAMP-GEF proteins, and/or which modulate the function or effects of the normal or mutant proteins, or to produce the CalDAG-GEF and/or cAMP-GEF proteins, fusion proteins, functional domains, antigenic determinants, and/or antibodies of the invention. Transformed cells may also be implanted into hosts, including humans, for therapeutic or other reasons. Preferred host cells include mammalian cells, including pure or mixed cell cultures, as well as bacterial, yeast, nematode,

insect and other invertebrate cells. For uses as described below, preferred cells also include embryonic stem cells, zygotes, gametes, and germ line cells.

In another series of embodiments, the present invention provides transgenic animal models of diseases or disorders associated with mutations in the CalDAG-GEF and/or cAMP-GEF genes. The animal may be essentially any non-human mammal, including rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates. In addition, invertebrate models, including nematodes and insects, may be used for certain applications. The animal models are produced by standard transgenic methods including microinjection, electroporation, transfection, or other forms of transformation of embryonic stem cells, zygotes, gametes, and germ line cells with vectors including genomic or cDNA fragments, minigenes, homologous recombination vectors, viral insertion vectors and the like. Suitable vectors include vaccinia virus, adenovirus, adeno-associated virus, retrovirus, liposome transport, neuraltropic viruses, and Herpes simplex virus. The animal models may include transgenic sequences comprising or derived from the CalDAG-GEF and/or cAMP-GEF genes, including normal and mutant sequences, intronic, exonic and untranslated sequences, and sequences encoding subsets of the CalDAG-GEF and/or cAMP-GEF proteins, such as functional domains. The major types of animal models provided include: (1) Animals in which a normal human CalDAG-GEF and/or cAMP-GEF gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a recombinant gene or a large genomic fragment; in which a normal human CalDAG-GEF and/or cAMP-GEF gene has been recombinantly substituted for one or both copies of the animal's homologous CalDAG-GEF and/or cAMP-GEF gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's homologous CalDAG-GEF and/or cAMP-GEF genes have been recombinantly "humanized" by the partial substitution of sequences encoding the human homologue by homologous recombination or gene targeting; (2) Animals in which a mutant human CalDAG-GEF and/or cAMP-GEF gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a recombinant gene or a large genomic fragment; in which a mutant human CalDAG-GEF and/or cAMP-GEF gene has been recombinantly substituted for one or both copies of the animal's homologous CalDAG-GEF and/or cAMP-GEF gene by homologous recombination or gene targeting; and/or in which one or both copies of one of the animal's

homologous CalDAG-GEF and/or cAMP-GEF gene have been recombinantly "humanized" by the partial substitution of sequences encoding a mutant human homologue by homologous recombination or gene targeting; (3) Animals in which a mutant version of one of that animal's CalDAG-GEF or cAMP-GEF gene has been recombinantly introduced into the genome of the animal as an additional gene, under the regulation of either an exogenous or an endogenous promoter element, and as either a recombinant gene or a large genomic fragment; and/or in which a mutant version of one of that animal's CalDAG-GEF or cAMP-GEF gene has been recombinantly substituted for one or both copies of the animal's homologous CalDAG-GEF or cAMP-GEF gene by homologous recombination or gene targeting; and (4) "Knock-out" animals in which one or both copies of one of the animal's CalDAG-GEF or cAMP-GEF genes have been partially or completely deleted by homologous recombination or gene targeting, or have been inactivated by the insertion or substitution by homologous recombination or gene targeting of exogenous sequences.

In another series of embodiments, the present invention provides for substantially pure protein preparations including polypeptides comprising or derived from the CalDAG-GEF and/or cAMP-GEF proteins. The CalDAG-GEF and cAMP-GEF protein sequences of the invention include the specifically disclosed sequences, variants of these sequences resulting from alternative mRNA splicing, allelic variants of these sequences, and homologous or orthologous variants of these sequences. Thus, for example, the invention provides amino acid sequences from the Mus musculus CalDAG-GEFI protein, Homo sapiens CalDAG-GEFI protein, Rattus norvegicus CalDAG-GEFII protein, Homo sapiens CalDAG-GEFII protein, Rattus norvegicus cAMP-GEFI protein, Homo sapiens cAMP-GEFI protein, Homo sapiens alternatively spliced cAMP-GEFI protein, Rattus norvegicus cAMP-GEFII protein, and Homo sapiens cAMP-GEFII protein. The present invention also provides allelic variants and homologous or orthologous proteins by providing methods by which such variants may be routinely obtained. The present invention also specifically provides for mutant or disease-causing variants of CalDAG-GEF and cAMP-GEF by providing methods by which such variants may be routinely obtained. Because the proteins of the invention may be used in a variety of diagnostic, therapeutic and recombinant applications, various subsets of the CalDAG-GEF and cAMP-GEF protein sequences and combinations of the CalDAG-GEF and cAMP-GEF protein sequences with heterologous sequences are also provided. For example, for use as immunogens or in binding assays, subsets of the CalDAG-GEF and cAMP-GEF protein sequences, including both normal and mutant

sequences, are provided. Such protein sequences may comprise a small number of consecutive amino acid residues from the sequences which are disclosed or otherwise enabled herein, but preferably include at least 4-8, and preferably at least 9-15 consecutive amino acid residues from a CalDAG-GEF or cAMP-GEF sequence. In another embodiment, such sequences comprise at least 15-200 consecutive amino acid residues from a CalDAG-GEF or cAMP-GEF sequence. Other preferred subsets of the CalDAG-GEF and cAMP-GEF protein sequences include those corresponding to one or more of the functional domains or antigenic determinants of the CalDAG-GEF and cAMP-GEF proteins and, in particular, may include either normal (wild-type) or mutant sequences. The invention also provides for various protein constructs in which a CalDAG-GEF and/or cAMP-GEF sequences, either complete or subsets thereof, are joined to exogenous sequences to form fusion proteins and the like. In accordance with these embodiments, the present invention also provides for methods of producing all of the above described proteins which comprise, or are derived from, CalDAG-GEF and/or cAMP-GEF.

In another series of embodiments, the present invention provides for the production and use of polyclonal and monoclonal antibodies, including antibody fragments, including Fab fragments, $F(ab')_2$, and single chain antibody fragments, which selectively bind to CalDAG-GEF or cAMP-GEF, or to specific antigenic determinants of CalDAG-GEF or cAMP-GEF. The antibodies may be raised in mouse, rabbit, goat or other suitable animals, or may be produced recombinantly in cultured cells such as hybridoma cell lines. Preferably, the antibodies selectively bind to a sequence comprising at least 4-8, and preferably at least 9-15, consecutive amino acid residues from a CalDAG-GEF or cAMP-GEF sequence. The antibodies of the invention may be used in the various diagnostic, therapeutic and technical applications described herein.

In another series of embodiments, the present invention provides methods of screening or identifying proteins, small molecules or other compounds which are capable of inducing or inhibiting the expression and/or function of the CalDAG-GEF and/or cAMP-GEF genes or proteins. The assays may be performed *in vitro* using non-transformed cells, immortalized cell lines, or recombinant cell lines, or *in vivo* using the transgenic animal models enabled herein. In particular, the assays may detect the presence of increased or decreased expression of CalDAG-GEF and/or cAMP-GEF-related genes or proteins on the basis of increased or decreased mRNA expression, increased or decreased levels of CalDAG-GEF and/or cAMP-GEF-related protein products, or increased or decreased levels of expression of a marker gene (*e.g.*, β-galactosidase,

green fluorescent protein, alkaline phosphatase or luciferase) operably joined to a 5' regulatory region in a recombinant construct. Cells known to express CalDAG-GEF or cAMP-GEF, or transformed to express CalDAG-GEF or cAMP-GEF, are incubated and one or more test compounds are added to the medium. After allowing a sufficient period of time (*e.g.*, 0-72 hours) for the compound to induce or inhibit the expression of the CalDAG-GEF or cAMP-GEF, any change in levels of expression from an established baseline may be detected using any of the techniques described above. In particularly preferred embodiments, the cells are from an immortalized cell line such as a human neuroblastoma, glioblastoma or a hybridoma cell line, or are transformed cells of the invention.

In another series of embodiments, the present invention provides methods for identifying proteins and other compounds which bind to, or otherwise directly interact with, CalDAG-GEF and/or cAMP-GEF. The proteins and compounds will include endogenous cellular components which interact with the CalDAG-GEF and/or cAMP-GEF *in vivo* and which, therefore, provide new targets for pharmaceutical and therapeutic interventions, as well as recombinant, synthetic, and otherwise exogenous compounds which may have CalDAG-GEF and/or cAMP-GEF binding capacity and, therefore, may be candidates for pharmaceutical agents. Thus, in one series of embodiments, cell lysates or tissue homogenates (*e.g.*, human brain homogenates, lymphocyte lysates) may be screened for proteins or other compounds which bind to one of the normal or mutant CalDAG-GEF or cAMP-GEF proteins. Alternatively, any of a variety of exogenous compounds, both naturally occurring and/or synthetic (*e.g.*, libraries of small molecules or peptides), may be screened for CalDAG-GEF or cAMP-GEF binding capacity. In each of these embodiments, an assay is conducted to detect binding between a "CalDAG-GEF component" or a "cAMP-GEF component" and some other moiety. In one embodiment, a CalDAG-GEF component comprises a CalDAG-GEF SRC1, SRC2, SRC3, EF hand or a DAG-binding domain. In another embodiment, a cAMP-GEF component comprises a cAMP-GEF SRC1, SRC2, SRC3, or a cAMP-binding domain. The "CalDAG-GEF component" or the "cAMP-GEF component" in these assays may be any polypeptide comprising or derived from a normal or mutant CalDAG-GEF or cAMP-GEF protein, including functional domains or antigenic determinants of CalDAG-GEF or cAMP-GEF, or CalDAG-GEF or cAMP-GEF fusion proteins. Binding may be detected by non-specific measures (*e.g.*, changes in intracellular Ca^{2+}, GTP/GDP ratio) or by specific measures (*e.g.*, changes in the expression of downstream genes which can be monitored by differential display, 2D gel electrophoresis, differential hybridization, or SAGE methods). The

preferred methods involve variations on the following techniques: (1) direct extraction by affinity chromatography; (2) co-isolation of CalDAG-GEF or cAMP-GEF components and bound proteins or other compounds by immunoprecipitation; (3) the Biomolecular Interaction Assay (BIAcore); and (4) the yeast two-hybrid systems.

In another series of embodiments, the present invention provides for methods of identifying proteins, small molecules and other compounds capable of modulating the activity of normal or mutant CalDAG-GEF or cAMP-GEF. Using normal cells or animals, the transformed cells and transgenic animal models of the present invention, or cells obtained from subjects bearing normal or mutant CalDAG-GEF or cAMP-GEF genes, the present invention provides methods of identifying such compounds on the basis of their ability to affect the expression of CalDAG-GEF and/or cAMP-GEF, the intracellular localization of the CalDAG-GEF and/or cAMP-GEF, or other biochemical, histological, or physiological markers which distinguish cells bearing normal and mutant CalDAG-GEF and/or cAMP-GEF sequences. Using the transgenic animals of the invention, methods of identifying such compounds are also provided on the basis of the ability of the compounds to affect behavioral, physiological or histological phenotypes associated with mutations in CalDAG-GEF and/or cAMP-GEF.

In another series of embodiments, the present invention provides methods and reagents for the screening and diagnosis of diseases or disorders associated with mutations in the CalDAG-GEF and/or cAMP-GEF genes. Screening and/or diagnosis can be accomplished by methods based upon the nucleic acids (including genomic and mRNA/cDNA sequences), proteins, and/or antibodies disclosed and enabled herein, including functional assays designed to detect failure or augmentation of the normal CalDAG-GEF and/or cAMP-GEF activity and/or the presence of specific new activities conferred by the mutant CalDAG-GEF and/or cAMP-GEF. Thus, for example, screens and diagnostics based upon CalDAG-GEF and/or cAMP-GEF proteins are provided which detect differences between mutant and normal CalDAG-GEF or cAMP-GEF in electrophoretic mobility, in proteolytic cleavage patterns, in molar ratios of the various amino acid residues, or in ability to bind specific antibodies. In addition, screens and diagnostics based upon nucleic acids (gDNA, cDNA or mRNA) are provided which detect differences in nucleotide sequences by direct nucleotide sequencing, hybridization using allele specific oligonucleotides, restriction enzyme digest and mapping (*e.g.*, RFLP, REF-SSCP), electrophoretic mobility (*e.g.*, SSCP, DGGE), PCR mapping, RNase protection, chemical mismatch cleavage, ligase-mediated detection, and various other methods. Other methods are

also provided which detect abnormal processing of CalDAG-GEF and/or cAMP-GEF or proteins reacting with CalDAG-GEF and/or cAMP-GEF, alterations in CalDAG-GEF and/or cAMP-GEF transcription, translation, and post-translational modification; alterations in the intracellular and extracellular trafficking of CalDAG-GEF and/or cAMP-GEF gene products; or abnormal intracellular localization of CalDAG-GEF and/or cAMP-GEF. Such methods and reagents are also useful in the analysis of neoplasias and mammalian immune system function, as well as functional *in vivo* imaging of mammalian organ systems. In accordance with these embodiments, diagnostic kits are also provided which will include the reagents necessary for the above-described diagnostic screens.

In another series of embodiments, the present invention provides methods and therapeutic agents for use in the treatment of conditions such as neurological and neuropsychiatric disorders such as Huntington's disease, Parkinson's disease, Alzheimer's disease, dystonia, Tourette's syndrome, obsessive-compulsive disorder, attention deficit/hyperactive disorder, depression, schizophrenia, and stroke; neoplasias such as solid tumors including colon, breast, lung, prostate, and hematopoietic tumors such as leukemia, Hodgkins, and non-Hodgkins lymphomas; and autoimmune diseases, allergies, and asthma; as well as for the enhancement of the immune response in normal and immunocompromised individuals. These methods and therapeutic agents may be based upon (1) administration of normal CalDAG-GEF and/or cAMP-GEF proteins; (2) gene therapy with normal CalDAG-GEF and/or cAMP-GEF genes to compensate for or replace the mutant genes; (3) gene therapy based upon antisense sequences to mutant CalDAG-GEF and/or cAMP-GEF genes or upon sequences which "knock-out" the mutant genes; (4) gene therapy based upon sequences which encode a protein which blocks or corrects the deleterious effects of CalDAG-GEF and/or cAMP-GEF mutants; (5) immunotherapy based upon antibodies to normal and/or mutant CalDAG-GEF and/or cAMP-GEF proteins; or (6) small molecules (drugs) which alter CalDAG-GEF and/or cAMP-GEF expression, block interactions between (normal or mutant) forms of CalDAG-GEF and/or cAMP-GEF and other proteins or ligands, or which otherwise block the function of (normal or mutant) CalDAG-GEF and/or cAMP-GEF proteins by altering the structure of the proteins, by enhancing their metabolic clearance, or by inhibiting their function.

In accordance with another aspect of the invention, the proteins of the invention can be used as starting points for rational drug design to provide ligands, therapeutic drugs or other types of small chemical molecules. Alternatively, small molecules or other compounds

identified by the above-described screening assays may serve as "lead compounds" in rational drug design.

Brief Description of the Drawings

Figure 1 is a partial schematic diagram of a Ras pathway.

Figure 2A shows human (h) and mouse (m) CalDAG-GEFI, human (h) and rat (r) CalDAG-GEFII, and *C. elegans* (cel) (F25B3.3, GenBank accession number: 1262950) CalDAG-GEF. Figure 2B shows a computer-generated phylogenetic tree analysis of the GEF domains of CalDAG-GEFI and CalDAG-GEFII in relation to other Ras-superfamily GEFs. Figure 2C shows multiple alignment of GEF structurally conserved regions (SCRs) of CalDAG-GEFs and several other GEFs of the Ras superfamily. Figure 2D shows the full-length amino acid sequences of human (h) and mouse (m) CalDAG-GEFI (box indicates amino acid differences). Figure 2E shows the sequence similarity (black indicates identity) of EF-hand domains in CalDAG-GEFs and other calcium binding proteins. Figure 2F shows the sequence similarity of DAG-binding domains of CalDAG-GEFs and PKC (protein kinase C) family proteins.

Figure 3A is a schematic representation of cAMP-GEF family proteins, including human (h) and rat (r) cAMP-GEFI, human (h) cAMP-GEFII and *C. elegans* (cel) (T2OG5.5, GenBank accession number: 458480) cAMP-GEF. Figure 3B is a phylogenetic tree analysis of cAMP binding domains of cAMP-GEFI and II and other cyclic nucleotide binding proteins. Figure 3C is a phylogenetic tree analysis of GEF domains of cAMP-GEFI and II and other Ras superfamily GEFs. Figure 3D shows the amino acid sequences of the structurally conserved regions (SCRs) of cAMP-GEFs and other Ras superfamily GEFs (black indicates identity). Figure 3E shows the amino acid sequences of the cAMP binding pockets of cAMP-GEFI and II and other cyclic nucleotide-binding proteins. The positions of invariant amino acid residues are shown by black diamonds. The open diamond indicates the amino acid that determines the binding specificity for cAMP or cGMP. The arrow indicates the position of amino acid substitutions specific to cAMP-GEFs. Figure 3F is the full-length amino acid sequences of human cAMP-GEFI and II (boxes indicate amino acid identity).

Detailed Description of the Invention

The present invention is based, in part, upon the discovery of a family of mammalian genes which are associated with the Ras pathway. The discovery of these genes, designated

CalDAG-GEFI, CalDAG-GEFII, cAMP-GEFI, and cAMP-GEFII, as well as the characterization of these genes, their protein products, mutants, and possible functional roles, are described below.

I. Definitions

In order to facilitate review of the various embodiments of the invention, and an understanding of the various elements and constituents used in making and using the invention, the following definitions are provided for particular terms used in the description and the claims which follow:

CalDAG-GEF. As used without further modification herein, the terms "CalDAG-GEF" or "CalDAG-GEFs" refer to the CalDAG-GEFI and/or the CalDAG-GEFII genes/proteins. In particular, the unmodified terms "CalDAG-GEF" or "CalDAG-GEFs" refer to the mammalian genes/proteins and, preferably, the human genes/proteins.

cAMP-GEF. As used without further modification herein, the terms "cAMP-GEF" or "cAMP-GEFs" refer to the cAMP-GEFI and/or the cAMP-GEFII genes/proteins. In particular, the unmodified terms "cAMP-GEF" or "cAMP-GEFs" refer to the mammalian genes/proteins and, preferably, the human genes/proteins.

CalDAG-GEF gene. As used herein, the term "CalDAG-GEF gene" means the mammalian genes represented by SEQ ID NOS: 1, 3, 5, and 7, as well as any allelic variants and heterospecific mammalian homologues. A murine CalDAG-GEFI cDNA sequence is disclosed herein as SEQ ID NO: 1, and a human CalDAG-GEFI cDNA sequence is disclosed herein as SEQ ID NO: 3. A rat CalDAG-GEFII cDNA sequence is disclosed herein as SEQ ID NO: 5, and a human CalDAG-GEFII cDNA sequence is disclosed herein as SEQ ID NO: 7. The term "CalDAG-GEF gene" primarily relates to a coding sequence, but can also include some or all of the flanking regulatory regions and/or introns. The term "CalDAG-GEF gene" specifically includes artificial or recombinant genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

CalDAG-GEF protein. As used herein, the term "CalDAG-GEF protein" means a protein encoded by a CalDAG-GEF gene, including allelic variants and heterospecific mammalian homologues. A murine CalDAG-GEFI protein sequence is disclosed herein as SEQ ID NO: 2, and a human CalDAG-GEFI protein sequence is disclosed herein as SEQ ID NO: 4. A rat CalDAG-GEFII protein sequence is disclosed herein as SEQ ID NO: 6, and a human CalDAG-GEFII protein sequence is disclosed herein as SEQ ID NO: 8. Splice variants are also embraced

by the term CalDAG-GEF protein as used herein. The protein may be produced by recombinant cells or organisms, may be substantially purified from natural tissues or cell lines, or may be synthesized chemically or enzymatically. Therefore, the term "CalDAG-GEF protein" is intended to include the protein in glycosylated, partially glycosylated, or unglycosylated forms, as well as in phosphorylated, partially phosphorylated, unphosphorylated, sulphated, partially sulphated, or unsulphated forms. The term also includes allelic variants and other functional equivalents of the CalDAG-GEF amino acid sequences, including biologically active proteolytic or other fragments.

hCalDAG-GEF gene and/or protein. As used herein, the abbreviation "hCalDAG-GEF" refers to the human homologue and human allelic variants of the CalDAG-GEF genes and/or proteins. Two cDNA sequences of the human CalDAG-GEF genes are disclosed herein as SEQ ID NOS: 3 and 7. The corresponding hCalDAG-GEF protein sequences are disclosed herein as SEQ ID NOS: 4 and 8. Allelic variants, including deleterious mutants, are enabled in the description which follows.

mCalDAG-GEF gene and/or protein. As used herein, the abbreviation "mCalDAG-GEF" refers to the murine homologues and murine allelic variants of the CalDAG-GEF gene and/or protein. A cDNA sequence of one murine CalDAG-GEF gene is disclosed herein as SEQ ID NO: 16. The corresponding mCalDAG-GEF protein sequence is disclosed herein as SEQ ID NO: 17. Allelic variants, including deleterious mutants, are enabled in the description which follows.

rCalDAG-GEF gene and/or protein. As used herein, the abbreviation "rCalDAG-GEF" refers to the rat homologue and rat allelic variants of the CalDAG-GEF genes and/or proteins. A cDNA sequence of one rat CalDAG-GEF gene is disclosed herein as SEQ ID NO: 5. The corresponding rCalDAG-GEF protein sequence is disclosed herein as SEQ ID NO: 6. Allelic variants, including deleterious mutants, are enabled in the description which follows.

cAMP-GEF gene. As used herein, the term "cAMP-GEF gene" means the mammalian genes represented by SEQ ID NOS: 9, 11, 13, 15, and 17, as well as any allelic variants and heterospecific mammalian homologues. A rat cAMP-GEFI cDNA sequence is disclosed herein as SEQ ID NO: 9, and a human cAMP-GEFI cDNA sequence is disclosed as SEQ ID NO:11. Another human cAMP-GEFI cDNA sequence, resulting from alternative splicing of the mRNA transcript, is disclosed as SEQ ID NO: 13. A rat cAMP-GEFII cDNA sequence is disclosed as SEQ ID NO: 15, and a human cAMP-GEFII cDNA sequence is disclosed as SEQ ID NO: 17.

The term "cAMP-GEF gene" primarily relates to a coding sequence, but can also include some or all of the flanking regulatory regions and/or introns. The term cAMP-GEF gene specifically includes artificial or recombinant genes created from cDNA or genomic DNA, including recombinant genes based upon splice variants.

cAMP-GEF protein. As used herein, the term "cAMP-GEF protein" means a protein encoded by a cAMP-GEF gene, including allelic variants and heterospecific mammalian homologues. A rat cAMP-GEFI protein sequence is disclosed herein as SEQ ID NO: 10, and a human cAMP-GEFI protein sequence is disclosed as SEQ ID NO: 12. Another human cAMP-GEFI protein sequence, resulting from alternative splicing of the mRNA transcript, is disclosed as SEQ ID NO: 14. A rat cAMP-GEFII protein sequence is disclosed as SEQ ID NO: 16, and a human cAMP-GEFII protein sequence is disclosed as SEQ ID NO: 18. Splice variants are also embraced by the term cAMP-GEF protein as used herein. The protein may be produced by recombinant cells or organisms, may be substantially purified from natural tissues or cell lines, or may be synthesized chemically or enzymatically. Therefore, the term "cAMP-GEF protein" is intended to include the protein in glycosylated, partially glycosylated, or unglycosylated forms, as well as in phosphorylated, partially phosphorylated, unphosphorylated, sulphated, partially sulphated, or unsulphated forms. The term also includes allelic variants and other functional equivalents of the cAMP-GEF amino acid sequences, including biologically active proteolytic or other fragments.

hcAMP-GEF gene and/or protein. As used herein, the abbreviation "hcAMP-GEF" refers to the human homologue and human allelic variants of the cAMP-GEF gene and/or protein. One cDNA sequences of the human cAMP-GEF gene is disclosed herein as SEQ ID NO: 18. The corresponding hcAMP-GEF protein sequence is disclosed herein as SEQ ID NO: 19. Numerous allelic variants, including deleterious mutants, are disclosed and enabled throughout the description which follows.

rcAMP-GEF gene and/or protein. As used herein, the abbreviation "rcAMP-GEF" refers to the rat homologue and rat allelic variants of the cAMP-GEF gene and/or protein. Two cDNA sequences of rat cAMP-GEF genes are disclosed herein as SEQ ID NOS: 9 and 15. The corresponding rcAMP-GEF protein sequences are disclosed herein as SEQ ID NOS: 10 and 16.. Numerous allelic variants, including deleterious mutants, are disclosed and enabled throughout the description which follows.

Normal. As used herein with respect to genes, the term "normal" refers to a gene which encodes and expresses a normal protein. As used herein with respect to proteins, the term "normal" means a protein which performs its usual or normal physiological role and which is not associated with, or causative of, a pathogenic condition or state. Therefore, as used herein, the term "normal" is essentially synonymous with the usual meaning of the phrase "wild type." For any given gene, or corresponding protein, a multiplicity of normal allelic variants may exist, none of which is associated with the development of a pathogenic condition or state. Such normal allelic variants include, but are not limited to, variants in which one or more nucleotide substitutions do not result in a change in the encoded amino acid sequence.

Mutant. As used herein with respect to genes, the term "mutant" refers to a gene which encodes a mutant protein and/or fails to express a normal protein. As used herein with respect to proteins, the term "mutant" means a protein which does not perform its usual or normal physiological role and which is associated with, or causative of, a pathogenic condition or state. Therefore, as used herein, the term "mutant" is essentially synonymous with the terms "dysfunctional," "pathogenic," "disease-causing," and "deleterious." With respect to the CalDAG-GEF and cAMP-GEF genes and proteins of the present invention, the term "mutant" refers to CalDAG-GEF and cAMP-GEF genes/proteins bearing one or more nucleotide/amino acid substitutions, insertions and/or deletions which cause the genes/proteins to be dysfunctional, pathogenic, disease-causing or otherwise deleterious. This definition is understood to include the various mutations that naturally exist, including but not limited to those disclosed herein, as well as synthetic or recombinant mutations produced by human intervention. The term "mutant," as applied to the CalDAG-GEF and cAMP-GEF genes, is not intended to embrace sequence variants which, due to the degeneracy of the genetic code, encode proteins identical to the normal sequences disclosed or otherwise enabled herein; nor is it intended to embrace sequence variants which, although they encode different proteins, encode proteins which are functionally equivalent to normal CalDAG-GEF and/or cAMP-GEF proteins.

Functional equivalent. As used herein in describing gene sequences and amino acid sequences, the term "functional equivalent" means that a recited sequence need not be identical to a particularly disclosed sequence of the SEQ ID NOs but need only provide a sequence which functions biologically and/or chemically as the equivalent of the disclosed sequence.

Substantially pure. As used herein with respect to protein preparations, the term "substantially pure" means a preparation which contains at least 60% (by dry weight) the protein

of interest, exclusive of the weight of other intentionally included compounds. Preferably the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by dry weight the protein of interest, exclusive of the weight of other intentionally included compounds. Purity can be measured by any appropriate method, e.g., column chromatography, gel electrophoresis, or HPLC analysis. If a preparation intentionally includes two or more different proteins of the invention, a "substantially pure" preparation means a preparation in which the total dry weight of the proteins of the invention is at least 60% of the total dry weight, exclusive of the weight of other intentionally included compounds. Preferably, for such preparations containing two or more proteins of the invention, the total weight of the proteins of the invention be at least 75%, more preferably at least 90%, and most preferably at least 99%, of the total dry weight of the preparation, exclusive of the weight of other intentionally included compounds. Thus, if the proteins of the invention are mixed with one or more other proteins (e.g., serum albumin, 6-OST) or compounds (e.g., diluents, detergents, excipients, salts, polysaccharides, sugars, lipids) for purposes of administration, stability, storage, and the like, the weight of such other proteins or compounds is ignored in the calculation of the purity of the preparation.

Isolated nucleic acid. As used herein, an "isolated nucleic acid" is a ribonucleic acid, deoxyribonucleic acid, or nucleic acid analog comprising a polynucleotide sequence that has been isolated or separated from sequences that are immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant nucleic acid which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (*e.g.*, a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequences and/or including exogenous regulatory elements.

Transformed cell. As used herein, a "transformed cell" is a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a nucleic acid molecule of interest. The nucleic acid of interest will typically encode a peptide or protein. The transformed cell may express the sequence of interest or may be used only to propagate the sequence. The term "transformed" may be used herein to embrace any method of introducing

exogenous nucleic acids including, but not limited to, transformation, transfection, electroporation, microinjection, viral-mediated transfection, and the like.

Operably joined. As used herein, a coding sequence and a regulatory region are said to be "operably joined" when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory region. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of promoter function results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the regulatory region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a regulatory region would be operably joined to a coding sequence if the regulatory region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide.

Stringent hybridization conditions. Stringent hybridization conditions is a term of art understood by those of ordinary skill in the art. For any given nucleic acid sequence, stringent hybridization conditions are those conditions of temperature, chaotrophic acids, buffer, and ionic strength which will permit hybridization of that nucleic acid sequence to its complementary sequence and not to substantially different sequences. The exact conditions which constitute "stringent" conditions, depend upon the nature of the nucleic acid sequence, the length of the sequence, and the frequency of occurrence of subsets of that sequence within other non-identical sequences. By varying hybridization conditions from a level of stringency at which non-specific hybridization occurs to a level at which only specific hybridization is observed, one of ordinary skill in the art can, without undue experimentation, determine conditions which will allow a given sequence to hybridize only with complementary sequences. Suitable ranges of such stringency conditions are described in KRAUSE ET AL., METHODS IN ENZYMOLOGY, 200: 546-56 (1991). Stringent hybridization conditions, depending upon the length and commonality of a sequence, may include temperatures of 20°C-65°C and ionic strengths from 5x to 0.1x SSC. Highly stringent hybridization conditions may include temperatures as low as 40-42°C (when denaturants such as formamide are included) or up to 60-65°C in ionic strengths as low as 0.1x SSC. These ranges, however, are only illustrative and, depending upon the nature of the target sequence, and possible future technological developments, may be more stringent than necessary.

Less than stringent conditions are employed to isolate nucleic acid sequences which are substantially similar, allelic or homologous to any given sequence.

Selectively bind. As used herein with respect to antibodies, an antibody is said to "selectively bind" to a target if the antibody recognizes and binds the target of interest but does not substantially recognize and bind other molecules in a sample, *e.g.*, a biological sample, which includes the target of interest.

CalDAG-GEF- or cAMP-GEF-associated disorder, condition, or disease. AS used herein, the term "CalDAG-GEF or cAMP-GEF associated disorder, condition, or disease" means any disorder, condition, or disease to which a normal or mutant CalDAG-GEF and/or cAMP-GEF is related in any manner, such as in the causation, prevention, exacerbation, alleviation of the disorder. Thus, as used herein, a CalDAG-GEF- or cAMP-GEF-associated disorder, condition, or disease includes disorders related to the Ras-pathway, such as Ras-related cancers.

Adapter protein. As used herein, the term "adapter protein" means any protein that binds or is bound to a CalDAG-GEF or a cAMP-GEF protein, and facilitates localization of the bound CalDAG-GEF or cAMP-GEF at the plasma membrane, thereby facilitating Ras activation.

Variant. As used herein a "variant" sequence has, or will result in having, a sufficient amino acid similarity to have a reasonable expectation of success in the methods of the present invention. In order to produce variants of the disclosed sequences that may also functionally serve as a CalDAG-GEF or cAMP-GEF protein, any one or more of the naturally-occurring CalDAG-GEF or cAMP-GEF sequences disclosed herein may be used as a reference sequence to determine whether a candidate sequence possesses sufficient amino acid similarity to have a reasonable expectation of success in the methods of the present invention. Preferably, variant sequences are at least 70% similar or 60% identical, more preferably at least 75% similar or 65% identical, and most preferably 80% similar or 70% identical to one of the disclosed, naturally-occurring sequences.

To determine whether a candidate peptide region has the requisite percentage similarity or identity to a reference polypeptide or peptide oligomer, the candidate amino acid sequence and the reference amino acid sequence are first aligned using the dynamic programming algorithm described in Smith and Waterman (1981), J. Mol. Biol. 147:195-197, in combination with the BLOSUM62 substitution matrix described in Figure 2 of Henikoff and Henikoff (1992), "Amino acid substitution matrices from protein blocks", PNAS (1992 Nov), 89:10915-10919. For the present invention, an appropriate value for the gap insertion penalty is -12, and an appropriate

value for the gap extension penalty is -4. Computer programs performing alignments using the algorithm of Smith-Waterman and the BLOSUM62 matrix, such as the GCG program suite (Oxford Molecular Group, Oxford, England), are commercially available and widely used by those skilled in the art.

Once the alignment between the candidate and reference sequence is made, a percent similarity score may be calculated. The individual amino acids of each sequence are compared sequentially according to their similarity to each other. If the value in the BLOSUM62 matrix corresponding to the two aligned amino acids is zero or a negative number, the pairwise similarity score is zero; otherwise the pairwise similarity score is 1.0. The raw similarity score is the sum of the pairwise similarity scores of the aligned amino acids. The raw score is then normalized by dividing it by the number of amino acids in the smaller of the candidate or reference sequences. The normalized raw score is the percent similarity. Alternatively, to calculate a percent identity, the aligned amino acids of each sequence are again compared sequentially. If the amino acids are non-identical, the pairwise identity score is zero; otherwise the pairwise identity score is 1.0. The raw identity score is the sum of the identical aligned amino acids. The raw score is then normalized by dividing it by the number of amino acids in the smaller of the candidate or reference sequences. The normalized raw score is the percent identity. Insertions and deletions are ignored for the purposes of calculating percent similarity and identity. Accordingly, gap penalties are not used in this calculation, although they are used in the initial alignment.

In all instances, variants of the naturally-occurring CalDAG-GEF or cAMP-GEF proteins, as described above, must be tested for biological activity as described below. Specifically, the proteins must exhibit guanine nucleotide exchange factor activity, and, preferably, they have the ability to inhibit Ras signaling of the Ras/Raf-1/MAP kinase pathway.

II. The CalDAG-GEFs

CalDAG-GEFI has a substrate specificity for Rap1A, dual binding domains for calcium (Ca^{2+}) and diacylglycerol (DAG), and enriched expression in brain basal ganglia pathways and their axon-terminal regions. Expression of CalDAG-GEFI activates Rap1A and inhibits Ras-dependent activation of the Erk/MAP kinase cascade in 293T cells. Ca^{2+} ionophore and phorbol ester strongly and additively enhance this Rap1A activation. By contrast, CalDAG-GEFII exhibits a different brain expression pattern and fails to activate Rap1A, but activates H-Ras, R-Ras and the Erk/MAP kinase cascade under Ca^{2+} and DAG modulation. The CalDAG-GEF

proteins have a critical neuronal function in determining the relative activation of Ras and Rap1 signaling induced by Ca^{2+} and DAG mobilization. The expression of CalDAG-GEFI and CalDAG-GEFII in hematopoietic organs indicates that such control has broad significance in Ras/Rap regulation of normal and malignant states.

The basal ganglia are centrally implicated in movement control and in forms of procedural learning related to habit formation. It is not yet known whether particular neurochemical specializations of the basal ganglia contribute to these behavioral functions. The basal ganglia do, however, have a unique double-inhibitory pathway design combined with abundant expression of neuromodulators in striatal neurons. A number of genes with differentially high expression in the striatum have also been identified. These include genes coding for proteins with signaling functions, such as adenylate cyclase V (Glatt et al., 361 NATURE (LONDON), 536-38 (1993)) and DARPP-32 (Hemmings et al., 310 NATURE (LONDON) 502-05 (1984)). To identify other cellular signaling molecules that could contribute to basal ganglia functions, a search for striatum-enriched transcripts was performed by a differential display method, as discussed in Example 1. Among the transcripts identified in this search were a family of genes characterized by the presence of a Ras superfamily (GEF) domain.

Specific domains identified include structurally conserved GEF regions SCR1, SCR2, and SCR3, as shown in Figures 2C and 3D, and as shown in the following table.

TABLE 1

Gene	SCR1	SCR2	SCR3
hCalDAG-GEFI	SEQ ID NO.3: 605-677 SEQ ID NO.4: 149-173	SEQ ID NO.3: 817-946 SEQ ID NO.4: 219-262	SEQ ID NO.3: 1053-1185 SEQ ID NO.4: 298-320
hCalDAG-GEFII	SEQ ID NO.7: 728-800 SEQ ID NO.8: 205-229	SEQ ID NO.7: 913-1042 SEQ ID NO.8: 270-313	SEQ ID NO.7: 1084-1216 SEQ ID NO.8: 348-371
hcAMP-GEFI	SEQ ID NO.11: 2058-2130 SEQ ID NO.12: 205-229	SEQ ID NO.11: 2276-2405 SEQ ID NO.12: 688-731	SEQ ID NO.11: 2516-2582 SEQ ID NO.12: 767-789
rcAMP-GEFI	SEQ ID NO.9: 2050-2122 SEQ ID NO.10: 618-642	SEQ ID NO.9: 2267-2396 SEQ ID NO.10: 691-734	SEQ ID NO.9: 2502-2568 SEQ ID NO.10: 770-792
hcAMP-GEFII	SEQ ID NO.17: 2707-2779 SEQ ID NO.18: 767-791		
rcAMP-GEFII	SEQ ID NO.15: 576-648 SEQ ID NO.16: 192-216		

In addition, the EF hand and DAG-binding domains were identified as shown in Figures 2E and 2F, and as shown in the following table:

Table 2

Gene	EF Hand Domain	DAG-Binding Domain
hCalDaG-GEFI	SEQ ID NO.3: 1456-1516 SEQ ID NO.4: 432-452	SEQ ID NO.3: 1652-1804 SEQ ID NO.4: 498-548
hCalDAG-GEFII	SEQ ID NO.7: 1384-1444 SEQ ID NO.8: 427-447	SEQ ID NO.7: 1579-1729 SEQ ID NO.8: 492-542

Finally, the cAMP-binding domains were identified as shown in Figure 3E, and as shown in the following table:

Table 3

Gene	cAMP-Binding Domain
hcAMP-GEFI	SEQ ID NO.11: 2012-2255 SEQ ID NO.12: 219-300
rcAMP-GEFI	SEQ ID NO.9: 853-1096 SEQ ID NO.10: 219-300
rcAMP-GEFII	SEQ ID NO.17: 1522-1765 SEQ ID NO.18: 372-453

III. The cAMP-GEFs

Cyclic adenosine 3', 5'-monophosphate (cAMP) is a universal second messenger that induces a variety of physiological responses in eukaryotic cells ranging from growth, differentiation, and gene expression to secretion and neurotransmission. The cAMP second messenger system has also been centrally implicated in modulating synaptic function, neuroplasticity and learning and memory. Most of these effects have been attributed to the binding of cAMP to cAMP-dependent protein kinase (PKA), leading in turn to the activation of

intracellular phosphorylation cascades. Reported herein is the identification of a new family of cAMP binding proteins that are differentially distributed in the brain and body organs and that are characterized by the presence of both a cAMP binding domain and a guanine nucleotide exchange factor (GEF) domain. These proteins, cAMP-GEFs, bind cAMP and selectively activate the Ras superfamily small G protein, Rap1A, in a cAMP-dependent but PKA-independent manner.

The general concept of cAMP signaling involves the sequential activation (or inhibition) of cAMP production by G proteins, the binding of cAMP to PKA, and the triggering of a series of downstream serine-threonine phosphorylation cascades. Viewed as the nearly exclusive target of cAMP binding in eukaryotic cells, PKA has been considered the essential effector molecule mediating a wide range of physiological effects of G protein/cAMP-triggered phosphorylation cascades. As the main cAMP effector, PKA has also been shown to function in the indirect coupling of the cAMP signal transduction system to other intracellular signaling cascades. The cAMP signaling system has also been strongly implicated in neuronal functions ranging from neurotransmitter-initiated signaling to neuroplasticity underlying development and memory, but PKA has not been clearly linked to all of these neuronal functions, and region-specific neuronal effects have been observed as well. The cAMP-GEF gene has a Ras superfamily GEF motif. Thus, the gene codes for a novel cAMP binding protein that directly couples the cAMP signal transduction system to Ras superfamily cascades.

IV. Preferred Embodiments

Based, in part, upon the discoveries disclosed and described herein, the following preferred embodiments of the present invention are provided.

1. Isolated Nucleic Acids

In one series of embodiments, the present invention provides isolated nucleic acids corresponding to, or relating to, the CalDAG-GEF or cAMP-GEF nucleic acid sequences disclosed herein. As described more fully below, these sequences include normal CalDAG-GEF and cAMP-GEF sequences from humans and other mammalian species, mutant CalDAG-GEF and cAMP-GEF sequences from humans and other mammalian species, homologous sequences from non-mammalian species such as *Drosophila* and *C. elegans*, subsets of these sequences useful as probes and PCR primers, subsets of these sequences encoding fragments of the CalDAG-GEF or cAMP-GEF proteins or corresponding to particular domains or polymorphic

regions, complementary or antisense sequences corresponding to fragments of the CalDAG-GEF or cAMP-GEF genes, sequences in which the CalDAG-GEF and/or cAMP-GEF coding regions have been operably joined to exogenous regulatory regions, and sequences encoding fusion proteins of the portions of the CalDAG-GEF or cAMP-GEF proteins fused to other proteins useful as markers of expression, as "tags" for purification, or in screens and assays for proteins interacting with the CalDAG-GEFs and/or cAMP-GEFs.

Thus, in a first series of embodiments, isolated nucleic acid sequences are provided which encode normal versions of the CalDAG-GEF and cAMP-GEF proteins. Examples of such nucleic acid sequences are disclosed herein. These nucleic acids may be genomic sequences or may be cDNA sequences (*e.g.*, SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, and 17). Thus, for example, the invention provides nucleic acid sequences in which the alternative splice variants described herein are incorporated at the DNA level, thereby, enabling cells including these sequences to express only one of the alternative splice variants at each splice position. For example, a recombinant gene may be produced in which one of the splice variants of cAMP-GEF is incorporated into DNA such that cells having this recombinant gene can express only one of these variants. For purposes of reducing the size of a recombinant CalDAG-GEF or cAMP-GEF gene, a cDNA gene may be employed or various combinations of the introns and untranslated exons may be removed from a DNA construct. Such constructs may be particularly useful, as described below, in identifying compounds which can induce or repress the expression of the CalDAG-GEF or cAMP-GEF genes.

In addition to the disclosed CalDAG-GEF and/or cAMP-GEF sequences, one of ordinary skill in the art is now enabled to identify and isolate nucleic acids corresponding to CalDAG-GEF or cAMP-GEF genes or cDNAs which are allelic to the disclosed sequences or which are heterospecific homologues. Thus, the present invention provides isolated nucleic acids corresponding to these alleles and homologues, as well as various recombinant constructs derived from these sequences, by means which are well known in the art. Briefly, one of ordinary skill in the art may now screen preparations of genomic or cDNA, including samples prepared from individual organisms (*e.g.*, human cancer patients or their family members) as well as bacterial, viral, yeast or other libraries of genomic or cDNA, using probes or PCR primers to identify allelic or homologous sequences. Because it is desirable to identify additional CalDAG-GEF and/or cAMP-GEF gene mutations which may contribute to the development of Ras-related cancers, because it is desirable to identify additional CalDAG-GEF and/or cAMP-

GEF polymorphisms which are not mutant or have antitumorigenic effects, and because it is also desired to create a variety of animal models which may be used to study Ras-related cancers and screen for potential therapeutics, it is particularly contemplated that additional CalDAG-GEF and/or cAMP-GEF sequences will be isolated from other preparations or libraries of human nucleic acids and from preparations or libraries from animals including rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates. Furthermore, CalDAG-GEF and/or cAMP-GEF homologues from yeast or invertebrate species, including *C. elegans* and other nematodes, as well as *Drosophila* and other insects, may have particular utility for drug screening. For example, invertebrates bearing mutant CalDAG-GEF and/or cAMP-GEF homologues (or mammalian CalDAG-GEF and/or cAMP-GEF transgenes) which cause a rapidly occurring and easily scored phenotype (*e.g.*, abnormal eye development after several days) can be used as screens for drugs which block the effect of the mutant gene. Such invertebrates may prove far more rapid and efficient for mass screenings than larger vertebrate animals. Once lead compounds are found through such screens, they may be tested in higher animals.

Depending upon the intended use, the present invention provides nucleic acid subsequences of the CalDAG-GEF and/or cAMP-GEF genes which may have lengths varying from 8-10 nucleotides (*e.g.*, for use as PCR primers) to nearly the full size of the CalDAG-GEF and/or cAMP-GEF genes. Thus, the present invention provides isolated nucleic acids comprising sequences corresponding to at least 8 , preferably at least 10, and more preferably at least 15 consecutive nucleotides of the CalDAG-GEF and/or cAMP-GEF genes, as disclosed or otherwise enabled herein, or to their complements.

In another series of embodiments, the present invention provides for isolated nucleic acids encoding all or a portion of the CalDAG-GEF and/or cAMP-GEF proteins in the form of a fusion protein. In these embodiments, a nucleic acid regulatory region (endogenous or exogenous) is operably joined to a first coding region which is covalently joined in-frame to a second coding region. The CalDAG-GEF and/or cAMP-GEF sequences of the fusion protein may represent the first, second, or any additional coding regions. The CalDAG-GEF and/or cAMP-GEF sequences may be conserved or non-conserved domains and can be placed in any coding region for the fusion protein.

In another series of embodiments, the present invention provides isolated nucleic acids in the form of recombinant DNA constructs in which a marker or reporter gene (*e.g.*, β-galactosidase, luciferase) is operably joined to the 5' regulatory region of a CalDAG-GEF and/or

cAMP-GEF gene such that expression of the marker gene is under the control of the CalDAG-GEF and/or cAMP-GEF regulatory sequences. Such isolated nucleic acids may be used to produce cells, cell lines or transgenic animals which are useful in the identification of compounds which can, directly or indirectly, differentially affect the expression of the CalDAG-GEFs and/or cAMP-GEFs.

Finally, the isolated nucleic acids of the present invention include any of the above described sequences when included in vectors. Appropriate vectors include cloning vectors and expression vectors of all types, including plasmids, phagemids, cosmids, episomes, and the like, as well as integration vectors. The vectors may also include various marker genes (*e.g.*, antibiotic resistance or susceptibility genes) which are useful in identifying cells successfully transformed therewith. In addition, the vectors may include regulatory sequences to which the nucleic acids of the invention are operably joined, and/or may also include coding regions such that the nucleic acids of the invention, when appropriately ligated into the vector, are expressed as fusion proteins. Such vectors may also include vectors for use in yeast "two hybrid," baculovirus, and phage-display systems.

2. Substantially Pure Proteins

The present invention provides for substantially pure preparations of the CalDAG-GEF and/or cAMP-GEF proteins, fragments of the CalDAG-GEF and/or cAMP-GEF proteins, and fusion proteins including the CalDAG-GEFs and/or cAMP-GEFs or fragments thereof. The proteins, fragments and fusions have utility, as described herein, in the generation of antibodies to normal and mutant CalDAG-GEFs and/or cAMP-GEFs, in the identification of CalDAG-GEF and/or cAMP-GEF binding proteins, and in diagnostic and therapeutic methods. Therefore, depending upon the intended use, the present invention provides substantially pure proteins or peptides comprising amino acid sequences which are subsequences of the complete CalDAG-GEF and/or cAMP-GEF proteins and which may have lengths varying from 4-8 amino acids (*e.g.*, for use as immunogens), or 9-15 amino acids (*e.g.*, for use in binding assays), to the complete CalDAG-GEF and/or cAMP-GEF proteins. Thus, the present invention provides substantially pure proteins or peptides comprising sequences corresponding to at least 4, preferably at least 9, more preferably at least 15 consecutive amino acids of the CalDAG-GEF and/or cAMP-GEF proteins, as disclosed or otherwise enabled herein.

Purification can be achieved using standard protein purification procedures including, but not limited to, gel-filtration chromatography, ion-exchange chromatography, high-performance

liquid chromatography (RP-HPLC, ion-exchange HPLC, size-exclusion HPLC, high-performance chromatofocusing chromatography, hydrophobic interaction chromatography, immunoprecipitation, or immunoaffinity purification. Gel electrophoresis (*e.g.*, PAGE, SDS-PAGE) can also be used to isolate a protein or peptide based on its molecular weight, charge properties, and hydrophobicity.

A CalDAG-GEF or cAMP-GEF protein, or a fragment thereof, may also be conveniently purified by creating a fusion protein including the desired CalDAG-GEF or cAMP-GEF sequence fused to another peptide such as an antigenic determinant or poly-His tag (*e.g.*, QIAexpress vectors, (QIAGEN Corp., Chatsworth, CA)), or a larger protein (*e.g.*, GST using the pGEX-27 vector (Amrad, USA) or green fluorescent protein using the Green Lantern vector (GIBCO/BRL. Gaithersburg, MD)).

3. Antibodies to the CalDAG-GEF and/or cAMP-GEFs

The present invention also provides antibodies, and methods of making antibodies, which selectively bind to the CalDAG-GEF and/or cAMP-GEF proteins or fragments thereof. The antibodies of the invention have utility as laboratory reagents for, *inter alia*, immunoaffinity purification of the CalDAG-GEFs and/or cAMP-GEFs, Western blotting to identify cells or tissues expressing the CalDAG-GEFs and/or cAMP-GEFs, and immunocytochemistry or immunofluorescence techniques to establish the subcellular location of the protein.

The antibodies of the invention may be generated in a host using the entire CalDAG-GEF and/or cAMP-GEF proteins of the invention or using any CalDAG-GEF and/or cAMP-GEF epitope which is characteristic of that protein and which substantially distinguishes it from host proteins. Such epitopes may be identified by comparing sequences of, for example, 4-8 amino acid residues from a CalDAG-GEF and/or cAMP-GEF sequence to computer databases of protein sequences from the relevant host. Antibodies against highly conserved domains are expected to have the greatest utility for purification or identification of CalDAG-GEFs and/or cAMP-GEFs.

Amino acid residue positions which are potential antigenic sites in the CalDAG-GEF or cAMP-GEF proteins and which may be useful in generating the antibodies of the invention may be determined by using computer programs such as the IBI Pustell program. Other methods of choosing antigenic determinants are known in the art and may, of course, be employed. In addition, larger fragments (*e.g.* 9-15 residues) including some of these epitopes may also be employed. Even larger fragments, including, for example, entire functional domains or multiple

functional domains may also be preferred. For an overview of antibody techniques, see Antibody Engineering: A Practical Guide, Borrebaek, ed., W.H. Freeman & Company, NY (1992), or Antibody Engineering, 2nd Ed., Borrebaek, ed., Oxford University Press, Oxford (1995).

The antibodies of the invention may be labelled or conjugated with other compounds or materials for diagnostic and/or therapeutic uses. For example, they may be coupled to radionuclides, fluorescent compounds, or enzymes for imaging or therapy, or to liposomes for the targeting of compounds contained in the liposomes to a specific tissue location.

4. Transformed Cell Lines

The present invention also provides for cells or cell lines, both prokaryotic and eukaryotic, which have been transformed or transfected with the nucleic acids of the present invention so as to cause clonal propagation of those nucleic acids and/or expression of the proteins or peptides encoded thereby. Such cells or cell lines will have utility not only in the propagation and production of the nucleic acids and proteins of the present invention but also, as further described herein, as model systems for diagnostic and therapeutic assays. As used herein, the term "transformed cell" is intended to embrace any cell, or the descendant of any cell, into which has been introduced any of the nucleic acids of the invention, whether by transformation, transfection, infection, electroporation, microinjection or other means. Methods of producing appropriate vectors, transforming cells with those vectors, and identifying transformants are well known in the art and are only briefly reviewed here (see, for example, Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Vectors may be introduced into the recipient or "host" cells by various methods well known in the art including, but not limited to, calcium phosphate transfection, strontium phosphate transfection, DEAE dextran transfection, electroporation, lipofection (*e.g.*, Dosper Liposomal transfection reagent, Boehringer Mannheim, Germany), microinjection, ballistic insertion on micro-beads, protoplast fusion or, for viral or phage vectors, by infection with the recombinant virus or phage.

5. Transgenic Animal Models

The present invention also provides for the production of transgenic non-human animal models for the study of Ras-related cancers, for the screening of candidate pharmaceutical compounds, for the creation of explanted mammalian cell cultures (*e.g.*, neuronal, glial,

organotypic or mixed cell cultures) in which mutant or wild type CalDAG-GEF and/or cAMP-GEF sequences are expressed or in which the CalDAG-GEF and/or cAMP-GEF genes have been inactivated (*e.g.*, "knock-out" deletions), and for the evaluation of potential therapeutic interventions.

Species suitable for use as animal models in the present invention include, but are not limited to, rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates (*e.g.*, Rhesus monkeys, chimpanzees).

Various techniques for generating transgenic animals, as well as techniques for homologous recombination or gene targeting, are now widely accepted and practiced. See, for example, Hogan et al., Manipulating Mouse Embryo (1986). To create a transgene, the target sequence of interest (*e.g.*, mutant or wild-type CalDAG-GEF or cAMP-GEF sequences) is typically ligated into a cloning site located downstream of a promoter element which will regulate the expression of RNA from the CalDAG-GEF or cAMP-GEF sequence. An alternate approach to creating a transgene is to use endogenous CalDAG-GEF or cAMP-GEF regulatory sequences to drive expression of the CalDAG-GEF or cAMP-GEF transgene.

6. Assays for Drugs Which Affect CalDAG-GEF and/or cAMP-GEF Expression

In another series of embodiments, the present invention provides assays for identifying small molecules or other compounds which are capable of inducing or inhibiting the expression of the CalDAG-GEF or cAMP-GEF genes and proteins. The assays may be performed *in vitro* using non-transformed cells, immortalized cell lines, or recombinant cell lines, or *in vivo* using the transgenic animal models enabled herein.

In particular, the assays may detect the presence of increased or decreased expression of CalDAG-GEF, cAMP-GEF, or other CalDAG-GEF or cAMP-GEF-related genes or proteins, on the basis of increased or decreased mRNA expression (using, *e.g.*, the nucleic acid probes disclosed and enabled herein), increased or decreased levels of CalDAG-GEF, cAMP-GEF or other CalDAG-GEF or cAMP-GEF-related protein products (using, *e.g.*, the anti-CalDAG-GEF of anti-cAMP-GEF antibodies disclosed and enabled herein), or increased or decreased levels of expression of a marker gene (*e.g.*, β-galactosidase or luciferase) operably joined to a CalDAG-GEF or cAMP-GEF 5' regulatory region in a recombinant construct.

Thus, for example, one may culture cells known to express a particular CalDAG-GEF or cAMP-GEF and add to the culture medium one or more test compounds. After allowing a sufficient period of time (*e.g.*, 0-72 hours) for the compound to induce or inhibit the expression

of the CalDAG-GEF or cAMP-GEF, any change in levels of expression from an established baseline may be detected using any of the techniques described above and well known in the art. In particularly preferred embodiments, the cells are from an immortalized cell line such as a human neuroblastoma, glioblastoma or a hybridoma cell line. Using the nucleic acid probes and/or antibodies disclosed and enabled herein, detection of changes in the expression of a CalDAG-GEF or cAMP-GEF and thus, identification of the compound as an inducer or repressor of CalDAG-GEF and/or cAMP-GEF expression, requires only routine experimentation.

In particularly preferred embodiments, a recombinant assay is employed in which a reporter gene such a β-galactosidase, green fluorescent protein, alkaline phosphatase, or luciferase is operably joined to a 5′ regulatory region of a CalDAG-GEF or cAMP-GEF gene. The reporter gene and regulatory regions are joined in-frame (or in each of the three possible reading frames) so that transcription and translation of the reporter gene may proceed under the control of the CalDAG-GEF or cAMP-GEF regulatory elements. The recombinant construct may then be introduced into any appropriate cell type, although mammalian cells are preferred, and human cells are most preferred. The transformed cells may be grown in culture and, after establishing the baseline level of expression of the reporter gene, test compounds may be added to the medium. The ease of detection of the expression of the reporter gene provides for a rapid, high through-put assay for the identification of inducers and repressors of the CalDAG-GEF or cAMP-GEF gene.

Compounds identified by this method will have potential utility in modifying the expression of the CalDAG-GEF, cAMP-GEF or other CalDAG-GEF or cAMP-GEF-related genes *in vivo*. These compounds may be further tested in the animal models disclosed and enabled herein to identify those compounds having the most potent *in vivo* effects. In addition, as described herein with respect to small molecules having CalDAG-GEF or cAMP-GEF-binding activity, these molecules may serve as "lead compounds" for the further development of pharmaceuticals by, for example, subjecting the compounds to sequential modifications, molecular modeling, and other routine procedures employed in rational drug design.

7. Identification of Compounds with CalDAG-GEF and/or cAMP-GEF Binding Capacity

In light of the present disclosure, one of ordinary skill in the art is enabled to practice new screening methodologies which will be useful in the identification of proteins and other compounds which bind to, or otherwise directly interact with, the CalDAG-GEFs or cAMP-GEFs. The proteins and compounds will include endogenous cellular components which interact

with the CalDAG-GEFs or cAMP-GEFs *in vivo* and which, therefore, provide new targets for pharmaceutical and therapeutic interventions, as well as recombinant, synthetic and otherwise exogenous compounds which may have CalDAG-GEF or cAMP-GEF binding capacity and, therefore, may be candidates for pharmaceutical agents. Thus, in one series of embodiments, cell lysates or tissue homogenates (*e.g.*, human brain homogenates, leukocyte lysates) may be screened for proteins or other compounds which bind to one of the normal or mutant CalDAG-GEFs and/or cAMP-GEFs. Alternatively, any of a variety of exogenous compounds, both naturally occurring and/or synthetic (*e.g.*, libraries of small molecules or peptides), may be screened for CalDAG-GEF or cAMP-GEF binding capacity. Small molecules are particularly preferred in this context because they are more readily absorbed after oral administration, have fewer potential antigenic determinants, and/or are more likely to cross the blood brain barrier than larger molecules such as nucleic acids or proteins. The methods of the present invention are particularly useful in that they may be used to identify molecules which selectively or preferentially bind to a mutant form of a CalDAG-GEF or cAMP-GEF protein (rather than a normal form) and, therefore, may have particular utility in treating the heterozygous victims of a CalDAG-GEF or cAMP-GEF associated disorder.

Compounds which bind to normal, mutant or both forms of the CalDAG-GEFs or cAMP-GEFs may have utility in treatments and diagnostics. Compounds which bind only to a normal CalDAG-GEF or cAMP-GEF may, for example, act as enhancers of its normal activity and thereby at least partially compensate for the lost or abnormal activity of mutant forms of the CalDAG-GEF or cAMP-GEF in victims suffering from CalDAG-GEF- or cAMP-GEF-associated disorders. Compounds which bind to both normal and mutant forms of a CalDAG-GEF or cAMP-GEF may have utility if they differentially affect the activities of the two forms so as to alleviate the overall departure from normal function. Alternatively, blocking the activity of both normal and mutant forms of either CalDAG-GEF or cAMP-GEF may have less severe physiological and clinical consequences than the normal progress of the disorder and, therefore, compounds which bind to and inhibit the activity of both normal and mutant forms of a CalDAG-GEF or cAMP-GEF may be therapeutically useful. Preferably, however, compounds are identified which have a higher affinity of binding to mutant CalDAG-GEF or cAMP-GEF than to normal CalDAG-GEF or cAMP-GEF, and which selectively or preferentially inhibit the activity of the mutant form. Such compounds may be identified by using any of the techniques

described herein, and then comparing the binding affinities of the candidate compound(s) for the normal and mutant forms of CalDAG-GEF or cAMP-GEF.

The effect of agents which bind to the CalDAG-GEFs or cAMP-GEFs (normal or mutant forms of either) can be monitored either by direct monitoring of this binding (*e.g.*, using the BIAcore assay, LKB Pharmacia, Sweden) or by indirect monitoring of binding by detecting, for example, a change in fluorescence, molecular weight, or concentration of either the binding agent or CalDAG-GEF or cAMP-GEF component, either in a soluble phase or in a substrate-bound phase.

Once identified by the methods described above, the candidate compounds may then be produced in quantities sufficient for pharmaceutical administration or testing (*e.g.*, μg or mg or greater quantities), and formulated in a pharmaceutically acceptable carrier (see, *e.g.*, REMINGTON'S PHARMACEUTICAL SCIENCES, Gennaro, A., ed., Mack Pub., (1990)). These candidate compounds may then be administered to the transformed cells of the invention, to the transgenic animal models of the invention, to cell lines derived from the animal models or from human patients, or to patients with CalDAG-GEF- or cAMP-GEF-associated disorders. The animal models described and enabled herein are of particular utility in further testing candidate compounds which bind to normal or mutant CalDAG-GEF or cAMP-GEF for their therapeutic efficacy.

In addition, once identified by the methods described above, the candidate compounds may also serve as "lead compounds" in the design and development of new pharmaceuticals. For example, as in well known in the art, sequential modification of small molecules (*e.g.*, amino acid residue replacement for peptides; functional group replacement for peptide or non-peptide compounds) is a standard approach in the pharmaceutical industry for the development of new pharmaceuticals. Such development generally proceeds from a "lead compound" which is shown to have at least some of the activity (*e.g.*, CalDAG-GEF or cAMP-GEF binding or blocking ability) of the desired pharmaceutical. In particular, when one or more compounds having at least some activity of interest (*e.g.*, modulation of CalDAG-GEF or cAMP-GEF activity) are identified, structural comparison of the molecules can greatly inform the skilled practitioner by suggesting portions of the lead compounds which should be conserved and portions which may be varied in the design of new candidate compounds. Thus, the present invention also provides a means of identifying lead compounds which may be sequentially modified to produce new candidate compounds for use in the treatment or CalDAG-GEF- or cAMP-GEF-associated

disorders. These new compounds then may be tested both for CalDAG-GEF or cAMP-GEF-binding or blocking (*e.g.*, in the binding assays described above) and for therapeutic efficacy (*e.g.*, in the animal models described herein). This procedure may be iterated until compounds having the desired therapeutic activity and/or efficacy are identified.

In each of the present series of embodiments, an assay is conducted to detect binding between a "CalDAG-GEF component" or a "cAMP-GEF component" and some other moiety. Of particular utility will be sequential assays in which compounds are tested for the ability to bind to only the normal or only the mutant forms of the CalDAG-GEF or cAMP-GEF functional domains using mutant and normal CalDAG-GEF or cAMP-GEF components in the binding assays. Such compounds are expected to have the greatest therapeutic utilities, as described more fully below. The "CalDAG-GEF component" or the "cAMP-GEF component" in these assays may be a complete normal or mutant form of a CalDAG-GEF or cAMP-GEF protein (*e.g.*, an hCalDAG-GEF or hcAMP-GEF variant) but need not be. Rather, particular functional domains of the CalDAG-GEFs or cAMP-GEFs, as described above, may be employed either as separate molecules or as part of a fusion protein. For example, to isolate proteins or compounds that interact with these functional domains, screening may be carried out using fusion constructs and/or synthetic peptides corresponding to these regions. Obviously, various combinations of fusion proteins and functional domains from CalDAG-GEF or cAMP-GEF are possible. In addition, the functional domains may be altered so as to aid in the assay by, for example, introducing into the functional domain a reactive group or amino acid residue (*e.g.*, cysteine) which will facilitate immobilization of the domain on a substrate (*e.g.*, using sulfhydryl reactions).

Methods for screening cellular lysates, tissue homogenates, or small molecule libraries for candidate CalDAG-GEF or cAMP-GEF-binding molecules are well known in the art and, in light of the present disclosure, may now be employed to identify compounds which bind to normal or mutant CalDAG-GEF or cAMP-GEF components or which modulate CalDAG-GEF or cAMP-GEF activity as defined by non-specific measures (*e.g.*, changes in intracellular Ca^{2+}, GTP/GDP ratio) or by specific measures (*e.g.*, changes in the expression of other downstream genes which can be monitored by differential display, 2D gel electrophoresis, differential hybridization, or SAGE methods). The preferred methods involve variations on the following techniques: (1) direct extraction by affinity chromatography; (2) co-isolation of CalDAG-GEF or cAMP-GEF components and bound proteins or other compounds by immunoprecipitation; (3)

the Biomolecular Interaction Assay (BIAcore); and (4) the yeast two-hybrid systems. These and others are discussed separately below.

A. Affinity Chromatography

In light of the present disclosure, a variety of affinity binding techniques well known in the art may be employed to isolate proteins or other compounds which bind to the CalDAG-GEFs or cAMP-GEFs disclosed or otherwise enabled herein. In general, a CalDAG-GEF or cAMP-GEF component may be immobilized on a substrate (*e.g.*, a column or filter) and a solution including the test compound(s) is contacted with the CalDAG-GEF or cAMP-GEF protein, fusion or fragment under conditions which are permissive for binding. The substrate is then washed with a solution to remove unbound or weakly bound molecules. A second wash may then elute those compounds which strongly bound to the immobilized normal or mutant CalDAG-GEF or cAMP-GEF component. Alternatively, the test compounds may be immobilized and a solution containing one or more CalDAG-GEF or cAMP-GEF components may be contacted with the column, filter, or other substrate. The ability of the CalDAG-GEF or cAMP-GEF component to bind to the test compounds may be determined as above or a labeled form of the CalDAG-GEF or cAMP-GEF component (*e.g.*, a radio-labeled or chemiluminescent functional domain) may be used to more rapidly assess binding to the substrate-immobilized compound(s).

B. Co-Immunoprecipitation

Another well characterized technique for the isolation of the CalDAG-GEF or cAMP-GEF components and their associated proteins or other compounds is direct immunoprecipitation with antibodies. This procedure has been successfully used, for example, to isolate many of the synaptic vesicle associated proteins (Phizicky et al., 59 J. BIOL. CHEM. 94-123 (1994)). Thus, either normal or mutant CalDAG-GEF or cAMP-GEF components may be mixed in a solution with the candidate compound(s) under conditions which are permissive for binding, and the CalDAG-GEF or cAMP-GEF component may be immunoprecipitated. Proteins or other compounds which co-immunoprecipitate with the CalDAG-GEF or cAMP-GEF component may then be identified by standard techniques as described above. General techniques for immunoprecipitation may be found in, for example, Harlow et al., ANTIBODIES: A LABORATORY MANUAL (1988).

The antibodies employed in this assay, as described and enabled herein, may be polyclonal or monoclonal, and include the various antibody fragments as well as single chain antibodies, and the like.

C. The Biomolecular Interaction Assay

Another useful method for the detection and isolation of binding proteins is the Biomolecular Interaction Assay or "BIAcore" system developed by Pharmacia Biosensor and described in the manufacturer's protocol (LKB Pharmacia, Sweden). In light of the present disclosure, one of ordinary skill in the art is now enabled to employ this system, or a substantial equivalent, to identify proteins or other compounds having CalDAG-GEF or cAMP-GEF binding capacity. The BIAcore system uses an affinity purified anti-GST antibody to immobilize GST-fusion proteins onto a sensor chip. Obviously, other fusion proteins and corresponding antibodies may be substituted. The sensor utilizes surface plasmon resonance which is an optical phenomenon that detects changes in refractive indices. A homogenate of a tissue of interest is passed over the immobilized fusion protein and protein-protein interactions are registered as changes in the refractive index. This system can be used to determine the kinetics of binding and to assess whether any observed binding is of physiological relevance.

D. The Yeast Two-Hybrid System

The yeast "two-hybrid" system takes advantage of transcriptional factors that are composed of two physically separable, functional domains. One commonly used system employs the yeast GAL4 transcriptional activator, consisting of a DNA binding domain and a transcriptional activation domain. Two different cloning vectors are used to generate separate fusions of the GAL4 domains to genes encoding potential binding proteins. The fusion proteins are co-expressed, targeted to the nucleus and, if interactions occur, activation of a reporter gene (*e.g.*, lacZ) produces a detectable phenotype.

E. Other Methods

The nucleotide sequences and protein products, including both mutant and normal forms of these nucleic acids and their corresponding proteins, can be used with the above techniques to isolate other interacting proteins, and to identify other genes whose expression is altered by the over-expression of normal CalDAG-GEF or cAMP-GEF sequences, by the under-expression of normal CalDAG-GEFs or cAMP-GEFs sequences, or by the expression of mutant CalDAG-GEF and/or cAMP-GEF sequences. Identification of these interacting proteins, as well as the identification of other genes whose expression levels are altered in the face of mutant CalDAG-

GEF or cAMP-GEF sequences (for instance) will identify other gene targets which have direct relevance to the pathogenesis of this disease in its clinical or pathological forms. Specifically, these techniques rely on PCR-based and/or hybridization-based methods to identify genes which are differentially expressed between two conditions (a cell line expressing normal CalDAG-GEFs or cAMP-GEFs compared to the same cell type expressing a mutant CalDAG-GEF or cAMP-GEF sequence). These techniques include differential display, serial analysis of gene expression (SAGE), mass-spectrometry of protein, 2D-gels and subtractive hybridization (*See, e.g.*, Nowak, 270 SCI. 368-371 (1995); Kahn, 270 SCI. 369-370 (1995)).

8. Methods of Identifying Compounds Modulating CalDAG-GEF and/or cAMP-GEF Activity

In another series of embodiments, the present invention provides for methods of identifying compounds with the ability to modulate the activity of normal and mutant CalDAG-GEFs and/or cAMP-GEFs. As used with respect to this series of embodiments, the term "activity" broadly includes gene and protein expression, CalDAG-GEF and/or cAMP-GEF protein post-translation processing, trafficking and localization, and any functional activity (*e.g.*, enzymatic, receptor-effector, binding, channel), as well as downstream affects of any of these. Using the transformed cells and transgenic animal models of the present invention, cells obtained from subjects bearing a mutant CalDAG-GEF and/or cAMP-GEF gene, or animals or human subjects bearing naturally occurring CalDAG-GEF and/or cAMP-GEF mutations, it is now possible to screen candidate pharmaceuticals and treatments for their therapeutic effects by detecting changes in one or more of the functional characteristics or phenotypic manifestations of normal or mutant CalDAG-GEF and/or cAMP-GEF expression.

Thus, the present invention provides methods for screening or assaying for proteins, small molecules or other compounds which modulate CalDAG-GEF and/or cAMP-GEF activity by contacting a cell *in vivo* or *in vitro* with a candidate compound and assaying for a change in a marker associated with normal or mutant CalDAG-GEF and/or cAMP-GEF activity. The marker associated with CalDAG-GEF and/or cAMP-GEF activity may be any measurable biochemical, physiological, histological and/or behavioral characteristic associated with CalDAG-GEF and/or cAMP-GEF expression. In particular, useful markers will include any measurable biochemical, physiological, histological and/or behavioral characteristic which distinguishes cells, tissues, animals or individuals bearing at least one mutant CalDAG-GEF and/or cAMP-GEF gene from their normal counterparts. In addition, the marker may be any specific or non-specific measure of CalDAG-GEF and/or cAMP-GEF activity, such as the GDP/GTP bound to Rap1/Ras.

CalDAG-GEF and/or cAMP-GEF specific measures include measures of CalDAG-GEF and/or cAMP-GEF expression (*e.g.*, CalDAG-GEF and/or cAMP-GEF mRNA or protein levels) which may employ the nucleic acid probes or antibodies of the present invention. Non-specific measures include changes in cell physiology such as pH. intracellular calcium, cAMP levels, overall GTP/GDP ratios, phosphatidylinositol activity, protein phosphorylation, etc.; which can be monitored by known methods. The activation or inhibition of CalDAG-GEF or cAMP-GEF activity in its mutant or normal form can also be monitored by examining changes in the expression of other genes which are specific to the CalDAG-GEF and/or cAMP-GEF pathway. These can be assayed by such techniques as differential display, differential hybridization, and SAGE, as well as by 2-D gel electrophoresis of cellular lysates. In each case, the differentially-expressed genes can be ascertained by inspection of identical studies before and after application of the candidate compound. Furthermore, as noted elsewhere, the particular genes whose expression is modulated by the administration of the candidate compound can be ascertained by cloning, nucleotide sequencing, amino acid sequencing, or mass spectrometry.

In general, a cell may be contacted with a candidate compound and, after an appropriate period (*e.g.*, 0-72 hours for most biochemical measures of cultured cells), the marker of CalDAG-GEF or cAMP-GEF activity may be assayed and compared to a baseline measurement. The baseline measurement may be made prior to contacting the cell with the candidate compound or may be an external baseline established by other experiments or known in the art. The cell may be a transformed cell of the present invention or an explant from an animal or individual. In particular, the cell may be an explant from a carrier of a CalDAG-GEF or cAMP-GEF mutation or an animal model of the invention (*e.g.*, a transgenic nematode or mouse bearing a mutant CalDAG-GEF or cAMP-GEF gene). Preferred cells include those from neurological tissues such as neuronal, glial or mixed cell cultures; and cultured fibroblasts, liver, kidney, spleen, or bone marrow. The cells may be contacted with the candidate compounds in a culture *in vitro* or may be administered *in vivo* to a live animal or human subject. For live animals or human subjects, the test compound may be administered orally or by any parenteral route suitable to the compound. For clinical trials of human subjects, measurements may be conducted periodically (*e.g.*, daily, weekly or monthly) for several months or years.

In light of the identification, characterization, and disclosure herein of the CalDAG-GEF or cAMP-GEF genes and proteins, the CalDAG-GEF or cAMP-GEF nucleic acid probes and antibodies, and the CalDAG-GEF or cAMP-GEF transformed cells and transgenic animals of the

invention, one of ordinary skill in the art is now enabled by perform a great variety of assays which will detect the modulation of CalDAG-GEF or cAMP-GEF activity by candidate compounds. Particularly preferred and contemplated embodiments are discussed in some detail below.

A. CalDAG-GEF and/or cAMP-GEF Expression

In one series of embodiments, specific measures of CalDAG-GEF or cAMP-GEF expression are employed to screen candidate compounds for their ability to affect CalDAG-GEF or cAMP-GEF activity. Thus, using the CalDAG-GEF or cAMP-GEF nucleic acids and antibodies disclosed and otherwise enabled herein, one may use mRNA levels or protein levels as a marker for the ability of a candidate compound to modulate CalDAG-GEF or cAMP-GEF activity. The use of such probes and antibodies to measure gene and protein expression is well known in the art and discussed elsewhere herein.

B. Intracellular Localization

In another series of embodiments, compounds may be screened for their ability to modulate the activity of the CalDAG-GEFs or cAMP-GEFs based upon their effects on the trafficking and intracellular localization of the CalDAG-GEFs or cAMP-GEFs. Differences in localization of mutant and normal CalDAG-GEFs and/or cAMP-GEFs may contribute to the etiology of CalDAG-GEF and/or cAMP-GEF-associated diseases. Compounds which can affect the localization of the CalDAG-GEFs and/or cAMP-GEFs may, therefore, be identified as potential therapeutics. Standard techniques known in the art may be employed to detect the localization of the CalDAG-GEFs and/or cAMP-GEFs. Generally, these techniques will employ the antibodies of the present invention, and in particular antibodies which selectively bind to one or more mutant CalDAG-GEFs or cAMP-GEFs but not to normal CalDAG-GEFs or cAMP-GEFs. As is well known in the art, such antibodies may be labeled by any of a variety of techniques (*e.g.*, fluorescent or radioactive tags, labeled secondary antibodies, avidin-biotin, etc.) to aid in visualizing the intracellular location of the CalDAG-GEFs or cAMP-GEFs. The CalDAG-GEFs or cAMP-GEFs may be co-localized to particular structures, as is known in the art, using antibodies to markers of those structures (*e.g.*, TGN38 for the Golgi, transferrin receptor for post-Golgi transport vesicles, LAMP2 for lysosomes). Western blots of purified fractions from cell lysates enriched for different intracellular membrane bound organelles (*e.g.*, lysosomes, synaptosomes, Golgi) may also be employed. In addition, the relative orientation of

different domains of the CalDAG-GEFs and/or cAMP-GEFs across cellular domains may be assayed using, for example, electron microscopy and antibodies raised to those domains.

9. Screening and Diagnostics for CalDAG-GEF- or cAMP-GEF-associated disorders

A. General Diagnostic Methods

The CalDAG-GEF or cAMP-GEF genes and gene products, as well as the CalDAG-GEF or cAMP-GEF-derived probes, primers and antibodies, disclosed or otherwise enabled herein, are useful in the screening for carriers of alleles associated with CalDAG-GEF- or cAMP-GEF-associated disorders. Individuals at risk for such a disorder or individuals not previously known to be at risk, may be routinely screened using probes to detect the presence of a mutant CalDAG-GEF or cAMP-GEF gene or protein by a variety of techniques. Diagnosis of inherited cases of these diseases can be accomplished by methods based upon the nucleic acids (including genomic and mRNA/cDNA sequences), proteins, and/or antibodies disclosed and enabled herein, including functional assays designed to detect increases or decreases of the normal CalDAG-GEF or cAMP-GEF activity and/or the presence of specific new activities conferred by the mutant CalDAG-GEFs or cAMP-GEFs. Preferably, the methods and products are based upon the human CalDAG-GEF or cAMP-GEF nucleic acids, proteins or antibodies, as disclosed or otherwise enabled herein. For brevity of exposition, but without limiting the scope of the invention, the following description will focus upon uses of the human homologues of CalDAG-GEF and cAMP-GEF. It will be understood, however, that homologous sequences from other species, including those disclosed herein, will be equivalent for many purposes.

B. Protein Based Screens and Diagnostics

When a diagnostic assay is to be based upon CalDAG-GEF or cAMP-GEF proteins, a variety of approaches are possible. For example, diagnosis can be achieved by monitoring differences in the electrophoretic mobility of normal and mutant proteins. Such an approach will be particularly useful in identifying mutants in which insertions, deletions or substitutions have resulted in a significant change in the electrophoretic migration of the resultant protein. Alternatively, diagnosis may be based upon differences in the proteolytic cleavage patterns of normal and mutant proteins, differences in molar ratios of the various amino acid residues, or by functional assays demonstrating altered function of the gene products.

C. Nucleic Acid Based Screens and Diagnostics

When the diagnostic assay is to be based upon nucleic acids from a sample, the assay may be based upon mRNA, cDNA or genomic DNA. Whether mRNA, cDNA, or genomic DNA is

assayed, standard methods well known in the art may be used to detect the presence of a particular sequence either *in situ* or *in vitro* (*See*, *e.g.*, Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed. (1989)).

(1) Appropriate Probes and Primers

Whether for hybridization, RNase protection, ligase-mediated detection, PCR amplification or any other standards methods described herein and well known in the art, a variety of subsequences of the CalDAG-GEF and/or cAMP-GEF sequences disclosed or otherwise enabled herein will be useful as probes and/or primers. These sequences or subsequences will include both normal CalDAG-GEF or cAMP-GEF sequences and deleterious mutant sequences. In general, useful sequences will include at least 8-10, more preferably 10-15, and most preferably 15-25 consecutive nucleotides from the CalDAG-GEF or cAMP-GEF introns, exons or intron/exon boundaries. In another embodiment, useful sequences include at least 25-500 consecutive nucleotides. Depending upon the target sequence, the specificity required, and future technological developments, shorter sequences may also have utility. Therefore, any CalDAG-GEF or cAMP-GEF derived sequence which is employed to isolate, clone, amplify, identify or otherwise manipulate a CalDAG-GEF or cAMP-GEF sequence may be regarded as an appropriate probe or primer.

(2) Hybridization Screening

For *in situ* detection of a normal or mutant CalDAG-GEF, cAMP-GEF or other CalDAG-GEF and/or cAMP-GEF-associated nucleic acid sequence, a sample of tissue may be prepared by standard techniques and then contacted with one or more of the above-described probes, preferably one which is labeled to facilitate detection, and an assay for nucleic acid hybridization is conducted under stringent conditions which permit hybridization only between the probe and highly or perfectly complementary sequences.

(3) Restriction Mapping

Sequence alterations may also create or destroy fortuitous restriction enzyme recognition sites which are revealed by the use of appropriate enzyme digestion followed by electrophoresis and visualization. DNA fragments carrying the site (normal or mutant) are detected by their increase or reduction in size, or by the increase or decrease of corresponding restriction fragment numbers. Such restriction fragment length polymorphism analysis (RFLP), or restriction mapping, may be employed with genomic DNA, mRNA or cDNA. The CalDAG-GEF or cAMP-GEF sequences may be amplified by PCR using the above-described primers prior to

restriction, in which case the lengths of the PCR products may indicate the presence or absence of particular restriction sites, and/or may be subjected to restriction after amplification. The CalDAG-GEF or cAMP-GEF fragments may be visualized by any convenient means (*e.g.*, under UV light in the presence of ethidium bromide).

(4) PCR Mapping

In another series of embodiments, a single base substitution mutation may be detected based on differential PCR product length or production in PCR. Thus, primers which span mutant sites or which, preferably, have 3' termini at mutation sites, may be employed to amplify a sample of genomic DNA, mRNA or cDNA from a subject. A mismatch at a mutational site may be expected to alter the ability of the normal or mutant primers to promote the polymerase reaction and, thereby, result in product profiles which differ between normal subjects and heterozygous and/or homozygous CalDAG-GEF or cAMP-GEF mutants.

(5) Electrophoretic Mobility

Genetic testing based on DNA sequence differences also may be achieved by detection of alterations in electrophoretic mobility of DNA, mRNA or cDNA fragments in gels. Small sequence deletions and insertions, for example, can be visualized by high resolution gel electrophoresis of single or double stranded DNA, or as changes in the migration pattern of DNA heteroduplexes in non-denaturing gel electrophoresis.

(6) Chemical Cleavage of Mismatches

Mutations in the CalDAG-GEFs or cAMP-GEFs may also be detected by employing the chemical cleavage of mismatch (CCM) method (*See, e.g.*, Saleeba et al., METHODS IN ENZYMOLOGY, 217: 286-295 (1993)). In this technique, probes (up to ~ 1 kb) may be mixed with a sample of genomic DNA, cDNA or mRNA obtained from a subject. The sample and probes are mixed and subjected to conditions which allow for heteroduplex formation (if any). Preferably, both the probe and sample nucleic acids are double-stranded, or the probe and sample may be PCR amplified together, to ensure creation of all possible mismatch heteroduplexes. Mismatched T residues are reactive to osmium tetroxide and mismatched C residues are reactive to hydroxylamine. Because each mismatched A will be accompanied by a mismatched T, and each mismatched G will be accompanied by a mismatched C, any nucleotide differences between the probe and sample (including small insertions or deletions) will lead to the formation of at least one reactive heteroduplex. After treatment with osmium tetroxide and/or hydroxylamine to modify any mismatch sites, the mixture is subjected to chemical cleavage at any modified

mismatch sites by, for example, reaction with piperidine. The mixture may then be analyzed by standard techniques such as gel electrophoresis to detect cleavage products which would indicate mismatches between the probe and sample.

(7) Other Methods

Various other methods of detecting CalDAG-GEF or cAMP-GEF mutations, based upon the CalDAG-GEF or cAMP-GEF sequences disclosed and otherwise enabled herein, will be apparent to those of ordinary skill in the art. Any of these may be employed in accordance with the present invention. These include, but are not limited to, nuclease protection assays (S1 or ligase-mediated), ligated PCR, denaturing gradient gel electrophoresis (DGGE; *see, e.g.,* Fischer et al., 80 PROC. NAT'L ACAD. SCI (USA), 1578-83 (1983)), restriction endonuclease fingerprinting combined with SSCP (REF-SSCP; *see, e.g.,* Liu et al., 18 BIOTECHNIQUES 470-79 (1995)), and the like.

D. Other Screens and Diagnostics

Diagnosis also can be made by observation of alterations in CalDAG-GEF or cAMP-GEF transcription, translation, and post-translational modification and processing as well as alterations in the intracellular and extracellular trafficking of CalDAG-GEF or cAMP-GEF gene products in the brain and peripheral cells. Such changes will include alterations in the amount of CalDAG-GEF or cAMP-GEF messenger RNA and/or protein, alteration in phosphorylation state, abnormal intracellular location/distribution, abnormal extracellular distribution, etc. Such assays will include: Northern Blots (with CalDAG-GEF or cAMP-GEF-specific and non-specific nucleotide probes), Western blots and enzyme-linked immunosorbent assays (ELISA) (with antibodies raised specifically to a CalDAG-GEF or a cAMP-GEF functional domain, including various post-translational modification states).

E. Screening and Diagnostic Kits

In accordance with the present invention, diagnostic kits are also provided which will include the reagents necessary for the above-described diagnostic screens. For example, kits may be provided which include antibodies or sets of antibodies which are specific to one or more mutant epitopes. These antibodies may, in particular, be labeled by any of the standard means which facilitate visualization of binding. Alternatively, kits may be provided in which oligonucleotide probes or PCR primers, as described above, are present for the detection and/or amplification of mutant CalDAG-GEF, cAMP-GEF or other CalDAG-GEF and/or cAMP-GEF-associated nucleotide sequences. Again, such probes may be labeled for easier detection of

specific hybridization. As appropriate to the various diagnostic embodiments described above, the oligonucleotide probes or antibodies in such kits may be immobilized to substrates and appropriate controls may be provided.

10. Methods of Treatment

The present invention now provides a basis for therapeutic intervention in diseases which are associated to the CalDAG-GEFs or cAMP-GEFs in that they are caused, prevented, exacerbated, or alleviated, or which may be caused, prevented, exacerbated, or alleviated, by the either normal or mutant CalDAG-GEFs or cAMP-GEFs. In considering the various therapies described below, it is understood that such therapies may be targeted at tissue other than the brain where CalDAG-GEF or cAMP-GEF are also expressed.

Therapies to treat CalDAG-GEF and/or cAMP-GEF-associated diseases may be based upon (1) administration of normal CalDAG-GEF or cAMP-GEF proteins, (2) gene therapy with normal CalDAG-GEF or cAMP-GEF genes to compensate for or replace the mutant genes, (3) gene therapy based upon antisense sequences to mutant CalDAG-GEF or cAMP-GEF genes or which "knock-out" the mutant genes, (4) gene therapy based upon sequences which encode a protein which blocks or corrects the deleterious effects of CalDAG-GEF or cAMP-GEF mutants, (5) immunotherapy based upon antibodies to normal and/or mutant CalDAG-GEF or cAMP-GEF proteins, or (6) small molecules (drugs) which alter CalDAG-GEF or cAMP-GEF expression, block abnormal interactions between mutant forms of CalDAG-GEF or cAMP-GEF and other proteins or ligands, or which otherwise block the aberrant function of mutant CalDAG-GEF or cAMP-GEF proteins by altering the structure of the mutant proteins, by enhancing their metabolic clearance, or by inhibiting their function.

A. Protein Therapy

Treatment of CalDAG-GEF and/or cAMP-GEF-associated disorders, or disorders resulting from CalDAG-GEF and/or cAMP-GEF mutations, may be performed by providing an excess of inactive mutant protein to decrease the effect of the normal function of the protein, or by providing an excess of normal protein to reduce the effect of any aberrant function of the mutant protein, by replacing a mutant protein with normal protein, or by modulating the function of the mutant protein.

B. Gene Therapy

In one series of embodiments, gene therapy may be employed in which normal or mutant copies of the CalDAG-GEF gene or the cAMP-GEF gene are introduced into patients to code

successfully for normal or mutant protein in one or more different affected cell types. The gene must be delivered to those cells in a form in which it can be taken up and code for sufficient protein to provide effective function. Thus, it is preferred that the recombinant gene be operably joined to a strong promoter so as to provide a high level of expression which will compensate for, or out-compete, the naturally-occurring proteins. As noted above, the recombinant construct may contain endogenous or exogenous regulatory elements, inducible or repressible regulatory elements, or tissue-specific regulatory elements.

In another series of embodiments, gene therapy may be employed to replace the naturally-occurring gene by homologous recombination with a recombinant construct. The recombinant construct may contain a normal or a mutant copy of the targeted CalDAG-GEF and/or cAMP-GEF gene, in which case the defect is corrected *in situ,* or may contain a "knock-out" construct which introduces a stop codon, missense mutation, or deletion which abolished function of the mutant gene. It should be noted in this respect that such a construct may knock-out both the normal and mutant copies of the targeted CalDAG-GEF and/or cAMP-GEF gene in a heterozygous individual, but the total loss of CalDAG-GEF and/or cAMP-GEF gene function may be less deleterious to the individual than continued progression of the disease state.

In another series of embodiments, antisense gene therapy may be employed. The antisense therapy is based on the fact that sequence-specific suppression of gene expression can be achieved by intracellular hybridization between mRNA or DNA and a complementary antisense species. The formation of a hybrid duplex may then interfere with the transcription of the gene and/or the processing, transport, translation and/or stability of the target CalDAG-GEF and/or cAMP-GEF mRNA. Antisense strategies may use a variety of approaches including the administration of antisense oligonucleotides or antisense oligonucleotide analogs (*e.g.*, analogs with phosphorothioate backbones) or transfection with antisense RNA expression vectors. Again, such vectors may include exogenous or endogenous regulatory regions, inducible or repressible regulatory elements, or tissue-specific regulatory elements.

In another series of embodiments, gene therapy may be used to introduce a recombinant construct encoding a protein or peptide which blocks or otherwise corrects the aberrant function caused by a naturally-occurring CalDAG-GEF and/or cAMP-GEF gene. In one embodiment, the recombinant gene may encode a peptide which corresponds to a mutant domain of a CalDAG-GEF and/or cAMP-GEF which has been found to abnormally interact with another cell protein or other cell ligand. Alternatively, the portion of a protein which interacts with a mutant, but not a

normal, CalDAG-GEF and/or cAMP-GEF may be encoded and expressed by a recombinant construct in order to compete with, and thereby inhibit or block, the aberrant interaction.

Retroviral vectors can be used for somatic cell gene therapy especially because of their high efficiency of infection and stable integration and expression. The targeted cells however must be able to divide and the expression of the levels of normal protein should be high. The full length CalDAG-GEF or cAMP-GEF genes, subsequences encoding functional domains of the CalDAG-GEFs or cAMP-GEFs, or any of the other therapeutic peptides described above, can be cloned into a retroviral vector and driven from its endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for the target cell type of interest. Other viral vectors which can be used include adeno-associated virus, vaccinia virus, bovine papilloma virus, or a herpes virus such as Epstein-Barr virus.

C. Immunotherapy

Antibodies may be raised to a mutant CalDAG-GEF or cAMP-GEF protein (or a portion thereof) and be administered to a patient to bind or block the mutant protein and prevent its deleterious effects. Alternatively, antibodies may be raised to specific complexes between mutant or wild-type CalDAG-GEF or cAMP-GEF and their interaction partners.

A further approach is to stimulate endogenous antibody production to the desired antigen. An immunogenic composition may be prepared as injectables, as liquid solutions or emulsions. The CalDAG-GEF or cAMP-GEF protein or other antigen may be mixed with pharmaceutically acceptable excipients compatible with the protein. Such excipients may include water, saline, dextrose, glycerol, ethanol and combinations thereof. The immunogenic composition and vaccine may further contain auxiliary substances such as emulsifying agents or adjuvants to enhance effectiveness. Immunogenic compositions and vaccines may be administered parenterally by injection subcutaneously or intramuscularly.

The immunogenic preparations and vaccines are administered in such amount as will be therapeutically effective, protective and immunogenic. Dosage depends on the route of administration and will vary according to the size of the host.

D. Small Molecule Therapeutics

As described and enabled herein, the present invention provides for a number of methods of identifying small molecules or other compounds which may be useful in the treatment of CalDAG-GEF- or cAMP-GEF-associated disorders. Thus, for example, the present invention provides for methods of identifying CalDAG-GEF or cAMP-GEF binding proteins and, in

particular, methods for identifying proteins or other cell components which bind to or otherwise interact with mutant CalDAG-GEFs or cAMP-GEFs but not with the normal CalDAG-GEFs or cAMP-GEFs. The invention also provides for methods of identifying small molecules which can be used to disrupt undesired interactions between CalDAG-GEFs or cAMP-GEFs and other proteins or other cell components.

By identifying these proteins and analyzing these interactions, it is possible to screen for or design compounds which counteract or prevent the interaction, thereby, providing treatment for abnormal interactions. Therapies can be designed to modulate these interactions and thereby, to modulate CalDAG-GEF- or cAMP-GEF-associated disorders. The potential efficacy of these therapies can be tested by analyzing the affinity and function of these interactions after exposure to the therapeutic agent by standard pharmacokinetic measurements of affinity (*e.g.*, Kd, Vmax) using synthetic peptides or recombinant proteins corresponding to functional domains of the CalDAG-GEF gene, the cAMP-GEF gene or other CalDAG-GEF and/or cAMP-GEF homologues. Another method for assaying the effect of any interactions involving functional domains is to monitor changes in the intracellular trafficking and post-translational modification of the relevant genes by *in situ* hybridization, immunohistochemistry, Western blotting and metabolic pulse-chase labeling studies in the presence of, and in the absence of, the therapeutic agents. A further method is to monitor the effects of "downstream" events including changes in second messenger events, *e.g.*, cAMP, intracellular Ca^{2+}, protein kinase activities, etc.

The effect of potential therapeutic agents in cell lines and whole animals can be monitored by monitoring transcription, translation, and post-translational modification of the CalDAG-GEF and/or cAMP-GEF proteins. Methods for these studies include Western and Northern blots, immunoprecipitation after metabolic labelling (pulse-chase) with radio-labelled methionine and ATP, and immunohistochemistry. The effect of these agents can also be monitored using studies which examine the relative binding affinities and relative amounts of CalDAG-GEF or cAMP-GEF proteins involved in interactions with Rap1A, using either standard binding affinity assays or co-precipitation and Western blots using antibodies to Rap1A, CalDAG-GEF, cAMP-GEF, or other CalDAG-GEF and/or cAMP-GEF homologues.

Therapy using antisense oligonucleotides to block the expression of the mutant CalDAG-GEF gene or the mutant cAMP-GEF gene, co-ordinated with gene replacement with normal CalDAG-GEF or cAMP-GEF gene can also be applied using standard techniques of either gene therapy or protein replacement therapy.

V. Examples

Example 1: Isolation and characterization of CalDAG-GEF.

Human full-length CalDAG-GEFI cDNAs were isolated from a human frontal cortex λZAPII cDNA library (Stratagene) and a U937 λZAPII cDNA library. Mouse full-length CalDAG-GEFI was identified in the mouse EST database (GenBank accession number: W71787). Rat full-length CalDAG-GEFII cDNA was isolated from a rat whole brain λZAPII cDNA library by using human CalDAG-GEFII as a probe. Mouse ESTs identified through BLAST searches were purchased from Genome Systems Inc. (St. Louis, MO).

CalDAG-GEFI encodes an approximately 69-kD protein (Fig. 2D) that displays in its amino terminal region a GEF domain that is highly homologous to Ras-superfamily GEFs (Fig. 2A-2D). Multiple alignment analysis shows that genes of the CalDAG-GEF family form a cluster within the Ras-GEF superfamily distinct from Ras GEFs such as Sos1 and rRas-GEF (Fig. 2B). The region downstream of the GEF domain contains two tandem repeats of EF-hand Ca^{2+} binding motifs (Figs. 2A, 2E). The carboxy-terminal region displays a typical diacylglycerol/phorbol ester-binding domain, which is present in most PKC family proteins (Fig. 2A, 2F). Multiple sequence alignments and phylogenetic tree analysis were carried out with the LASERGENE Software Package (DNASTAR Inc.). Abbreviations and GenBank accession numbers of the protein sequences used in Figure 2 are as follows: C3G: 474982, mCdc25: 882120, rRas-GRF: 57665, hSos1 (human son-of-sevenless 1): 476780, BUD5: 171141, hCalmodulin: 115512, hCalbindin D28k: 227666, hCalcineurin B: 105504, hParvalbumin a: 131100, hTroponin C: 136043, hPKCa: 125549, hPKCb1: 125538, hPKCg: 462455.

To determine the small G protein target of CalDAG-GEFI, guanine nucleotide exchange activity *in vivo* was analyzed using intact 293T cells cotransfected with a eukaryotic expression construct of mouse CalDAG-GEFI and GST-tagged Ras family proteins. Full-length mouse CalDAG-GEFI cDNA inserted into a pCMV-SPORT expression vector with a carboxy-terminal FLAG epitope was used for transfection. A PCR-amplified fragment of rat CalDAG-GEFII was subcloned into a pCAGGS expression vector with the addition of His_6-tag at its amino-terminus, resulting in pCAGGS-His-CalDAGII. pEBG-Krev1 that expresses Rap1A was used as a fusion protein to glutathione S-transferase (GST) in mammalian cells, as described in Gotoh et al., 15 Mol. Cell Biol. 6746-53 (1995), pEBG-R-Ras, other vectors for Ras-family proteins obtained by inserting PCR-amplified cDNAs into pEBG expression vector, pCAGGS-C3G and pCAGGS-MSos1, and pCEV-H-RasV12. CalDAG-GEFI transfection produced a dramatic increase in

GTP-bound Rap1A compared to the control but showed no or minimal activation of H-Ras, R-Ras, or Ral A. The increase in GTP-bound Rap1A was augmented in the presence of either the Ca^{2+} ionophore, A23187, or the phorbol ester, phorbol-12-myristate-13-acetate (TPA). Further, A23187 and TPA had additive effects when administered together.

To determine the effect of CalDAG-GEFI on the Erk/MAP kinase cascade, Elk1 activation was measured in 293T cells transfected with CalDAG-GEFI or constitutively active H-Ras (RasV12), or both. 293T cells were transfected by SuperFect (Qiagen) as described in Gotoh, *supra*, with expression vectors for GST-tagged Ras family proteins and with those for various GEFs. Cells were labeled 24 hours after transfection with $^{32}P_i$ for 2 hr. In some experiments, cells were stimulated with either 10 μM A23187 or 1 μM phorbol-12-myristate-13-acetate (TPA) for 3 min. GST-tagged Ras family proteins were collected from cell lysates with glutathione Sepharose. Guanine nucleotides bound to Ras family proteins were separated by thin layer chromatography (TLC). Activation of Elk1 was examined by the PathDetect Elk1 transreporting system (Stratagene). 293T cells were transfected with pFR-Luc and pFA-Elk1 with various expression vectors, and light output was detected and analyzed by the use of LAS1000 film. CalDAG-GEFI reduced RasV12 activation of Elk1 by approximately 4-fold and did not itself activate Elk1. Thus, CalDAG-GEFI strongly inhibits Ras-dependent stimulation of the Erk/MAP kinase cascade.

Northern analysis showed that human CalDAG-GEFI is expressed strongly in the brain and that CalDAG-GEFI mRNA is strikingly enriched in the striatum. Probes used included human CalDAG-GEFI: 729-bp EcoRI fragment, human CalDAG-GEFII: 584-bp SacI and HindIII fragment, rat CalDAG-GEFI: 439-bp fragment of EST clone RBC565 (GenBank accession number: C06861, and rat CalDAG-GEFII: 508-bp PCR amplified and subcloned fragment (nucleic acids 2541 to 3048 of SEQ ID NO:5). *In situ* hybridization of sections from the adult rat brain confirmed these restricted distribution patterns. Intense signal was present in the striatum (caudoputamen) and the ventral striatum (nucleus accumbens, olfactory tubercle). There was weaker signal in the olfactory bulb.

A series of monoclonal antibodies against the carboxy-terminal half of mouse CalDAG-GEFI were raised. His_6-tagged mouse CalDAG-GEFI polypeptide (amino acids 349 to 608 of SEQ ID NO:1) was expressed in bacteria, purified over Ni^{2+}-nitrilotriacetic acid-agarose resin, and then used to immunize BALB/c mice. The resultant polyclonal antiserum was monitored by ELISA, Western blot, immunoprecipitation, and immunofluorescence assays on CalDAG-GEFI-

transfected COS-7 cells. Hybridomas were generated by PEG (polyethylene glycol)-mediated fusion of donor splenocytes to the SP2/O cell line. Positive hybridoma cell lines were identified by screening in the assays described above, and purified by limiting dilution and single-cell cloning. Three hybridoma cell lines against mouse CalDAG-GEFI (mAbs 18B11, 2D9, and 18A7), in addition to the polyclonal fusion serum, were identified. Western analysis showed that mAbs 18B11 and 2D9 were specific for CalDAG-GEFI. Lightly post-fixed, cryostat-cut 10 μm thick sections were immunostained by the ABC (Vectastain kit) method for CalDAG-GEFI with mAbs 18B11 and 2D9 and the polyclonal fusion serum, for tyrosine hydroxylase (TH) with monoclonal antibodies from INCSTAR, and for μ opioid receptor with polyclonal antiserum. Immunohistochemistry with mAb 18B11 showed a striking basal ganglia-enriched distribution pattern in sections of adult rat brain, with significant but weaker activity elsewhere. CalDAG-GEFI immunoreactivity marked the entire pathway from the striatal matrix compartment to the pallidum and substantia nigra pars reticulata, where very intense CalDAG-GEFI staining was present. Thus, CalDAG-GEFI is synthesized in striatal projection neurons and is transported to striatopallidal and striatonigral terminals.

To confirm that CalDAG-GEFI is synthesized in striatal projection neurons and transported to striatopallidal and striatonigral terminals in rats, intrastriatal injections of ibotenic acid (20 μg/μl, 1.5 μl per site, 5 day survival) were made unilaterally at 2 sites in the mid-lateral caudoputamen, with contralateral vehicle control injections were made. In other rats, unilateral subthalamic knife-cuts were made at an anteroposterior level between the entopeduncular nucleus and substantia nigra to sever the striatonigral efferents (1 and 3 days survivals), with control contralateral thalamic knife-cuts. These procedures all reduced CalDAG-GEFI staining in the substantia nigra. *In situ* hybridization was performed according to Simmons et al, 12 J. Histotechnol. 169-181 (1989). A 439bp rat EST clone RBC565 (98.4% identical to mouse CalDAG-GEFI nucleic acids 1777 to 2216 of SEQ ID NO:1) was isolated by BLAST search and used for making RNA probes with ^{32}P-labeled UTP (2,000 Ci/mmol, NEN, 1 Ci = 37 GBq) and T3 and T7 RNA polymerase. Brains were processed as above for CalDAG-GEFI and TH immunostaining. Thus, CalDAG-GEFI is a protein transported in striatal axons to their terminals. The terminal localization of CalDAG-GEFI was confirmed in subcellular fractionation experiments on dissected samples from the rat ventral midbrain, in which Western analysis showed the presence of CalDAG-GEFI in cytosol and in membrane fractions, including synaptosomes.

Because of the similarity of the GEF domains of CalDAG-GEFI and CalDAG-GEFII, the substrate specificity of CalDAG-GEFII with the same 293T cell assay system used for CalDAG-GEFI was examined. It was confirmed that CalDAG-GEFII activates Ras, and further shown that it activates H-Ras and R-Ras, but not Ral A or Rap1A. H-Ras activation was enhanced by A23187 and TPA. Moreover, CalDAG-GEFII, unlike CalDAG-GEFI, increased the transcriptional activity of Elk1 downstream to Erk/MAP kinase. Thus, in the 293T system, CalDAG-GEFI and CalDAG-GEFII target different Ras-superfamily small G proteins and have opposite effects on the MAP kinase cascade. Northern analysis further showed contrasting brain expression for CalDAG-GEFII, with highest expression being in the cerebellum, cerebral cortex, and amygdala, and low expression occurring in the striatum. Both genes are also expressed in hematopoietic organs in both human and rat.

Rap signaling is important in regulating basal ganglia output in response to Ca^{2+} and DAG. Corticostriatal inputs can activate the MAP kinase cascade in striatal projection neurons (Sgambat et al., 18 J. Neurosci. 214-26 (1993)) and phosphoinositide (PI) signaling is strongly represented in these pathways (Fotuhi et al., 13 J. Neurosci. 3300-08 (1993)). Moreover, a number of receptor systems in the striatum and its striatonigral/striatopallidal pathways are linked to Ca^{2+} and PI signaling, notably including NMDA and metabotropic glutamate receptors, D_2-class dopamine receptors, and tachykinin receptors (Fiorillo et al., 394 Nature 78-82 (1998)). A previously unrecognized signaling target for some of these systems is likely to be Rap1, via CalDAG-GEFI. In addition, CalDAG-GEFI has a synaptic function as demonstrated by the heavy accumulation of CalDAG-GEFI in the target nuclei of striatal outputs and the localization of Rap1 in synaptosomes and synaptic vesicles. The particular basal ganglia projection systems are enriched in CalDAG-GEFI and are differentially vulnerable to neurodegeneration in Huntington's disease.

Rap and Ras functions can be regulated coordinately or disjunctively by Ca^{2+} and DAG in the brain and hematopoietic organs, depending on the relative expression of CalDAG-GEFI and CalDAG-GEFII. In neurons, Ras/MAP kinase signaling has been directly implicated in synaptic transmission and the neuroplasticity underlying learning and memory. Different CalDAG-GEFI and CalDAG-GEFII expression patterns in the brain influence region-specific neuroplasticity mediated by Ca^{2+} and DAG signaling pathways. The presence of CalDAG-GEFI and CalDAG-GEFII in the hematopoietic system demonstrates the direct input of Ca^{2+} and DAG to Ras/Rap regulation of normal growth and differentiation as well as malignant transformation.

Example 2: Isolation and characterization of cAMP-GEFs.

cAMP-GEFI and cAMP-GEFII have similar domain structures, with a cAMP binding domain at the amino terminus and a GEF domain at the carboxy terminus separated by a link region (LR) (Fig. 3A). These mammalian proteins show strong structural homology to a predicted open reading frame (T20G5.5) in *C. elegans* cAMP-GEF (cel cAMP-GEF) (Fig. 3A). The cAMP binding domains of the cAMP-GEF family proteins form a distinct group within the cyclic nucleotide-binding protein superfamily and show the closest similarity to the B domains of PKA regulatory subunits (Fig. 3B). A PR(A/T)AT motif in the cAMP binding pocket is also conserved in the cAMP-GEF proteins (Fig. 3E). The first alanine of this motif confers cAMP (alanine) as opposed to cGMP (threonine) binding specificity. All of the cAMP-GEF family members have alanine at this position, and therefore bind cAMP rather then cGMP.

The GEF domains of the cAMP-GEFs show high homology to those of Ras-GEF family proteins, but form an independent cluster distinct from Ras GEFs such as mCdc25, hSos1, and rRas-GRF (Fig. 3, C and D). The three structurally conserved regions specific to Ras-GEF family proteins (SCR1, SCR2, and SCR3) are present in all of the cAMP-GEF proteins (Fig. 3D). Multiple sequence alignments and phylogenetic tree analyses were carried out with LASERGENE (DNASTAR Inc.). Abbreviations and GenBank accession numbers of the protein sequences used in this figure: hPKARIα (human cAMP-dependent protein kinase regulatory subunit type I-alpha): 125193, hPKARIβ: 1346362, hPKARIIα: 125198, hPKARIIβ: 400115, hPKGIα (human cGMP-dependent protein kinase type I-alpha): 1255602, hPKGIβ: 125379, hPKGII: 1906312, hCalDAG-GEFI: U71870, hCalDAG-GEFII: AF081195, C3G: 474982, hSosl (human son-of-sevenless 1): 476780, mCdc25: 882120, rRas-GRF: 57665, BUD5: 171141.

In order to identify the small G protein substrate for cAMP-GEFI and II and the mode of cAMP regulation of GEF activity conferred by these proteins, the effects of cAMP-GEFI and cAMP-GEFII expression were analyzed in 293T cells on the ratio of GTP to GDP bound to different Ras family small G proteins in the presence or absence of forskolin and IBMX. Under basal conditions, in the absence of forskolin and IBMX, only Rap1 was activated significantly. In the presence of forskolin and IBMX, both cAMP-GEFI and II strongly and selectively activated Rap1A, but did not activate H-Ras, R-Ras or RalA. The effects of forskolin/IBMX treatment on cAMP-GEFI and II were dose-dependent with EC_{50} values of 1.8 μM and 0.3 μM, respectively. Forskolin/IBMX treatment given alone had no effect.

A time-course analysis of the activation of Rap1A by forskolin/IBMX in cAMP-GEFI transfectants showed that the activation began within 10 sec, reached a maximum at 5 min, and continued for at least 60 min. Thus, cAMP-GEFI has a direct effect on Rap1A rather than secondary effects mediated by other Ras-superfamily GEFs. In addition, Sp-cAMPS, an analogue of cAMP, activated Rap1A at levels similar to those induced by forskolin/IBMX. Thus, cAMP has the capacity to activate the GEF domain of cAMP-GEFI.

Mutational analyses with cAMP-GEFI was performed to examine whether its cAMP-binding domain is required for the activation of Rap1A. In contrast to wild type cAMP-GEFI, a deletion mutant lacking a cAMP binding domain (pcDNA-rcAMP-GEFI:DcAMP(528) and (595)) did not activate Rap1A with or without forskolin/IBMX treatment. Mutants with a single amino acid substitution at the cAMP binding pocket (pcDNA-rcAMP-GEFI:R(279)K) responded minimally to forskolin/IBMX treatment. Thus, the cAMP binding domain of cAMP-GEFI is necessary for its cAMP-dependent activation of Rap1A.

To assess further the cAMP binding capacity of cAMP-GEFI, a cAMP agarose affinity bead binding assay was performed. *In vitro* translated, radiolabeled cAMP-GEFI showed selective binding to the beads that was competed by excess amounts of either cAMP or 8-Br-cAMP. cAMP-GEF protein can bind cAMP and that this binding can activate Rap1A.

cAMP-dependent activation of Rap1 has previously been ascribed to the phosphorylation of Rap1A by PKA, which raises its affinity to smgGDS, a GEF with broad substrate specificity. However, at least in the 293T cell assay system, an increase of GTP-bound Rap1A in response to increasing cAMP levels with forskolin or treatment with the cAMP analogue, Sp-cAMPS was not detected in the absence of cAMP-GEFs. In addition, even in the presence of H-89, a potent and selective inhibitor of PKA, cAMP-GEFI and II could still activate Rap1A. The activation of Rap1A induced by cAMP-GEFI and II is independent of the PKA pathway.

Intracellular cAMP has been shown to interact directly with ion channels, but the vast majority of cAMP-mediated effects in eukaryotes have been considered as sequels to cAMP binding by the regulatory subunits of the PKA tetramer. The diversity of physiological effects produced by cAMP have been attributed to the fact that, as a kinase, PKA has a large range of molecular targets. Reported herein are novel cAMP binding proteins that directly link the cAMP second messenger system to Ras superfamily signaling pathways and that appear selectively to target Rap.

cAMP can exert profound cell-type specific effects on cell growth and differentiation and that cAMP is capable of inhibiting or stimulating the Ras/mitogen-activated protein (MAP) kinase/Erk pathway. The inhibition can occur at the initial translocation step by which Ras activates Raf, whereas activation of Rap1 is thought to occur through phosphorylation by PKA. Activation of Rap1 has been suggested to be part of a switch mechanism determining whether growth or differentiation occurs in response to nerve growth factor (NGF). cAMP-GEFs directly couple cAMP to Rap1, itself discovered as a negative regulator of Ras but suspected of having independent functions as well. Thus, different levels of cAMP-GEF expression confer cell-type specific regulation of Ras superfamily signaling systems.

The genes also exhibit developmentally regulated expression in the septum, medial thalamus and habenula, key structures in the limbic system variously linked to brain reward circuits, addiction and schizophrenia. Thus, cAMP-GEFs, in addition to PKA, underlie some of the neuronal functions of cAMP.

Example 3. Northern hybridization demonstrating the expression of CalDAG-GEFI and CalDAG-GEFII protein mRNAs in a variety of tissues.

Total cytoplasmic RNA was isolated from various human tissue samples including amygdala, cerebellum, corpus callosum, caudate nucleus, cortex, frontal lobe, hippocampus, kidney, liver, lung, medulla obongata, occipital pole, putamen, spinal cord, substantia nigra, subthalamic nucleus, thalamus, and temporal lobe, obtained from surgical pathology using standard procedures such as CsCl purification. The RNA was then electrophoresed on a formaldehyde gel to permit size fractionation. The nitrocellulose membrane was prepared and the RNA was then transferred onto the membrane. ^{32}P-labeled cDNA probes were prepared and added to the membrane in order for hybridization between the probe the RNA to occur. After washing, the membrane was wrapped in plastic film and placed into imaging cassettes containing X-ray film. The autoradiographs were then allowed to develop for one to several days. Sizing was established by comparison to standard RNA markers. These northern blots demonstrated that the CalDAG-GEF genes are strongly expressed in the brain. Weaker hybridization was detectable elsewhere.

Example 4. Northern hybridization demonstrating the expression of cAMP-GEFI and cAMP-GEFII protein mRNAs in a variety of tissues.

Northern hybridization analysis was performed as in Example 3 to detect the expression of the cAMP-GEFI and cAMP-GEFII genes in a variety of human tissues. The tissues analyzed included adrenal gland, amygdala, bone marrow, cerebellum, corpus callosum, caudate nucleus, colon (mucosal lining), caudputamen, cortex, frontal lobe, hippocampus, habenula, heart, kidney, liver, lung, lymph node, medulla obongata, occipital pole, olfactory bulb, ovary, pons, pancreas, putamen, septum, small intestines, skeletal muscle, spinal cord, spleen, stomach, substantia nigra, subthalamic nucleus, testis, thalamus, temporal lobe, thymus, trachea, and thyroid.

A striking contrast in the expression patterns of human cAMP-GEFI and II was observed by Northern analysis. Human cAMP-GEFI is widely expressed, with highest levels appearing in kidney, spleen, thyroid, heart, and pancreas. Human cAMP-GEFII shows a remarkably selective enrichment in the brain and the adrenal glands. Both genes were developmentally regulated. The expression patterns of the two genes in the nervous system also differ, with cAMP-GEFI having a wider expression than cAMP-GEFII. These region-specific neuronal expression patterns were confirmed in *in situ* hybridization experiments. cAMP-GEFI mRNA is expressed broadly at low levels in the adult brain, but it is strongly and selectively expressed in parts of the neonatal brain, including the septum and the thalamus. By contrast, cAMP-GEFII is strongly expressed in the mature as well as the developing brain. Notable are the high levels of cAMP-GEFII mRNA in the cerebral cortex, the hippocampus (especially CA3 and dentate gyrus), the habenula and the cerebellum. Genes of the cAMP-GEF family have widespread influence on cAMP functions in bodily organs and also region-specific functions in the brain.

Example 5. Isolation of CalDAG-GEF or cAMP-GEF binding proteins by yeast two-hybrid system.

To identify proteins interacting with the CalDAG-GEF or cAMP-GEF proteins, a yeast expression plasmid vector (pAS2-1, Clontech) is generated by ligating an in-frame partial cDNA sequence encoding either residues of the CalDAG-GEF protein or residues of the cAMP-GEF protein into the EcoRI and BamHI sites of the vector. The resultant fusion protein contains the GAL4 DNA binding domain coupled in-frame either to residues of the CalDAG-GEF protein or to residues of the cAMP-GEF protein. These expression plasmids are co-transformed, along with purified plasmid DNA from the human brain cDNA:pACT library, into yeast using the protocols of the Clontech Matchmaker yeast-two-hybrid kit (Clontech). Yeast clones bearing human brain cDNAs which interact with the CalDAG-GEF or cAMP-GEF fragments are selected by HIS resistance and βgal+ activation. The clones are further selected by

cyclohexamide sensitivity and the inserts of the human brain cDNAs are isolated by PCR and sequenced.

Although preferred embodiments of the invention have been described herein in detail, it will be understood by those skilled in the art that variations may be made thereto without departing from the spirit of the invention or the scope of the following claims.

CLAIMS

What is claimed is:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a protein selected from the group consisting of a normal CalDAG-GEFI protein, a mutant CalDAG-GEFI protein, a normal CalDAG-GEFII protein, and a mutant CalDAG-GEFII protein.

2. An isolated nucleic acid comprising a nucleotide sequence encoding a protein selected from the group consisting of a normal cAMP-GEFI protein, a mutant cAMP-GEFI protein, a normal cAMP-GEFII protein, and a mutant cAMP-GEFII protein.

3. An isolated nucleic acid as in claim 1 wherein said nucleic acid encodes a normal CalDAG-GEF protein and wherein said nucleotide sequence is selected from the group consisting of

(a) a sequence encoding a protein comprising the human CalDAG-GEFI amino acid sequence of SEQ ID NO: 4;

(b) a sequence encoding a protein comprising the murine CalDAG-GEFI amino acid sequence of SEQ ID NO: 2;

(c) a sequence encoding a protein comprising the human CalDAG-GEFII amino acid sequence of SEQ ID NO: 8; and

(d) a sequence encoding a protein comprising the murine CalDAG-GEFII amino acid sequence of SEQ ID NO: 6; and

(e) a sequence encoding a normal CalDAG-GEF protein and capable of hybridizing to a sequence complementary to any sequence of (a) - (d) under stringent hybridization conditions.

4. An isolated nucleic acid as in claim 2 wherein said nucleic acid encodes a normal cAMP-GEF protein and wherein said nucleotide sequence is selected from the group consisting of

(a) a sequence encoding a protein comprising the human cAMP-GEFI amino acid sequence of SEQ ID NO: 12;

(b) a sequence encoding a protein comprising the alternatively spliced human cAMP-GEFI amino acid sequence of SEQ ID NO: 14;

(c) a sequence encoding a protein comprising the rat cAMP-GEFI amino acid sequence of SEQ ID NO: 10;

(d) a sequence encoding a protein comprising the human cAMP-GEFII amino acid sequence of SEQ ID NO: 18;

(e) a sequence encoding a protein comprising the rat cAMP-GEFII amino acid sequence of SEQ ID NO: 16; and

(f) a sequence encoding a normal cAMP-GEF protein and capable of hybridizing to a sequence complementary to any sequence of (a) - (e) under stringent hybridization conditions.

5. An isolated nucleic acid comprising a nucleotide sequence of at least 8 consecutive nucleotides selected from the group consisting SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO. 17, and a sequence complementary to any of these sequences.

6. An isolated nucleic acid comprising a nucleotide sequence of at least 10 consecutive nucleotides selected from the group consisting SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO. 17, and a sequence complementary to any of these sequences.

7. An isolated nucleic acid comprising a nucleotide sequence of at least 15 consecutive nucleotides selected from the group consisting SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO. 17, and a sequence complementary to any of these sequences.

8. An isolated nucleic acid comprising a nucleotide sequence encoding at least one functional domain of a CalDAG-GEF protein selected from the group consisting of a normal CalDAG-GEFI protein, a mutant CalDAG-GEFI protein, a normal CalDAG-GEFII protein, and a mutant CalDAG-GEFII protein.

9. An isolated nucleic acid comprising a nucleotide sequence encoding at least one functional domain of a cAMP-GEF protein selected from the group consisting of a normal cAMP-GEFI protein, a normal cAMP-GEFII protein, a mutant cAMP-GEFI protein, and a mutant cAMP-GEFII protein.

10. An isolated nucleic acid comprising a nucleotide sequence encoding an antigenic determinant of a CalDAG-GEF protein selected from the group consisting of a normal CalDAG-GEFI protein, a normal CalDAG-GEFII protein, a mutant CalDAG-GEFI protein, and a mutant CalDAG-GEFII protein.

11. An isolated nucleic acid comprising a nucleotide sequence encoding an antigenic determinant of a cAMP-GEF protein selected from the group consisting of a normal cAMP-GEFI protein, a normal cAMP-GEFII protein, a mutant cAMP-GEFI protein, and a mutant cAMP-GEFII protein.

12. A method for identifying an allelic variant or heterospecific homologue of a human CalDAG-GEF gene comprising:

choosing a nucleic acid probe or primer capable of hybridizing to a human CalDAG-GEF gene sequence under stringent hybridization conditions;

mixing said probe or primer with a sample of nucleic acids which may contain a nucleic acid corresponding to said variant or homologue; and

detecting hybridization of said probe or primer to said nucleic acid corresponding to said variant or homologue.

13. A method as in claim 12 wherein said human CalDAG-GEF gene sequence is selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 7.

14. A method as in claim 12 wherein said sample comprises a sample of nucleic acids selected from the group consisting of human genomic DNA, human mRNA, and human cDNA.

15. A method as in claim 12 wherein said sample comprises a sample of nucleic acids selected from the group consisting of mammalian genomic DNA, mammalian mRNA, and mammalian cDNA.

16. A method as in claim 12 wherein said sample comprises a sample of nucleic acids selected from the group consisting of invertebrate genomic DNA, invertebrate mRNA, and invertebrate cDNA.

17. A method as in claim 12 further comprising the step of isolating said nucleic acid corresponding to said variant or homologue.

18. A method as in claim 12 wherein said nucleic acid is identified by hybridization.

19. A method as in claim 12 wherein said nucleic acid is identified by PCR amplification.

20. A method for identifying allelic variants or heterospecific homologues of a human cAMP-GEF gene comprising:

choosing a nucleic acid probe or primer capable of hybridizing to a human cAMP-GEF gene sequence under stringent hybridization conditions;

mixing said probe or primer with a sample of nucleic acids which may contain a nucleic acid corresponding to said variant or homologue; and

detecting hybridization of said probe or primer to said nucleic acid corresponding to said variant or homologue.

21. A method as in claim 12 wherein said human cAMP-GEF gene sequence is selected from the group consisting of SEQ ID NO: 11. SEQ ID NO: 13, and SEQ ID NO: 17.

22. A method as in claim 20 wherein said sample comprises a sample of nucleic acids selected from the group consisting of human genomic DNA, human mRNA, and human cDNA.

23. A method as in claim 20 wherein said sample comprises a sample of nucleic acids selected from the group consisting of mammalian genomic DNA, mammalian mRNA, and mammalian cDNA.

24. A method as in claim 20 wherein said sample comprises a sample of nucleic acids selected from the group consisting of invertebrate genomic DNA, invertebrate mRNA, and invertebrate cDNA.

25. A method as in claim 20 further comprising the step of isolating said nucleic acid corresponding to said variant or homologue.

26. A method as in claim 20 wherein said nucleic acid is identified by hybridization.

27. A method as in claim 20 wherein said nucleic acid is identified by PCR amplification.

28. A method for identifying an allelic variant or heterospecific homologue of a human CalDAG-GEF gene comprising:

choosing an antibody capable of selectively binding to a human CalDAG-GEF protein;

mixing said antibody with a sample of proteins which may contain a protein corresponding to said variant or homologue; and

detecting binding of said antibody to said protein corresponding to said variant or homologue.

29. A method as in claim 28 wherein said sample comprises a sample of proteins selected from the group consisting of human proteins, human fusion proteins, and proteolytic fragments thereof.

30. A method as in claim 28 wherein said sample comprises a sample of nucleic acids selected from the group consisting of mammalian proteins, mammalian fusion proteins, and proteolytic fragments thereof.

31. A method as in claim 28 wherein said sample comprises a sample of nucleic acids selected from the group consisting of invertebrate proteins, invertebrate fusion proteins, and proteolytic fragments thereof.

32. A method as in claim 28 further comprising the step of substantially purifying said protein corresponding to said variant or homologue.

33. A method for identifying an allelic variant or heterospecific homologue of a human cAMP-GEF gene comprising:

choosing an antibody capable of selectively binding to a human cAMP-GEF protein;

mixing said antibody with a sample of proteins which may contain a protein corresponding to said variant or homologue; and

detecting binding of said antibody to said protein corresponding to said variant or homologue.

34. A method as in claim 33 wherein said sample comprises a sample of proteins selected from the group consisting of human proteins, human fusion proteins, and proteolytic fragments thereof.

35. A method as in claim 33 wherein said sample comprises a sample of proteins selected from the group consisting of mammalian proteins, mammalian fusion proteins, and proteolytic fragments thereof.

36. A method as in claim 33 wherein said sample comprises a sample of proteins selected from the group consisting of invertebrate proteins, invertebrate fusion proteins, and proteolytic fragments thereof.

37. A method as in claim 33 further comprising the step of substantially purifying said protein corresponding to said variant or homologue.

38. An isolated nucleic acid comprising an allelic variant or a heterospecific homologue of a gene selected from the group consisting of a human CalDAG-GEF gene, and a human cAMP-GEF gene.

39. An isolated nucleic acid encoding an allelic variant or heterospecific homologue of a protein selected from the group consisting of a human CalDAG-GEF protein, and a human cAMP-GEF protein.

40. An isolated nucleic acid comprising a recombinant vector including a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, and a sequence complementary to any of these sequences.

41. An isolated nucleic acid as in claim 40 wherein said vector is an expression vector and said nucleotide sequence is operably joined to a regulatory region.

42. An isolated nucleic acid as in claim 41 wherein said expression vector may express said nucleotide sequence in mammalian cells.

43. An isolated nucleic acid as in claim 42 wherein said cells are selected from the group consisting of fibroblast, liver, kidney, spleen, bone marrow, and neurological cells.

44. An isolated nucleic acid as in claim 42 wherein said vector is selected from the group consisting of vaccinia virus, adenovirus, retrovirus, neurotropic viruses, and Herpes simplex.

45. An isolated nucleic acid as in claim 41 wherein said expression vector encodes at least a functional domain of a protein selected from the group consisting of normal CalDAG-GEFI, a normal CalDAG-GEFII, a mutant CalDAG-GEFI, a mutant CalDAG-GEFII, a normal cAMP-GEFI, a normal cAMP-GEFII, a mutant cAMP-GEFI, and a mutant cAMP-GEFII.

46. An isolated nucleic acid as in claim 41 wherein said vector further comprises sequences encoding an exogenous protein operably joined to said nucleotide sequence and whereby said vector encodes a fusion protein.

47. An isolated nucleic acid as in claim 46 wherein said exogenous protein is selected from the group consisting of lacZ, trpE, maltose-binding protein, poly-His tags, and glutathione-S-transferase.

48. An isolated nucleic acid comprising a recombinant expression vector including nucleotide sequences corresponding to an endogenous regulatory region of a gene selected from the group consisting of a CalDAG-GEF gene, and a cAMP-GEF gene.

49. An isolated nucleic acid as in claim 48 wherein said endogenous regulatory region is operably joined to a marker gene.

50. A host cell transformed with an expression vector of any one of claims 41-49, or a descendant thereof.

51. A host cell as in claim 50 wherein said host cell is selected from the group consisting of bacterial cells and yeast cells.

52. A host cell as in claim 50 wherein said host cell is selected from the group consisting of fetal cells, embryonic stem cells, zygotes, gametes, and germ line cells.

53. A host cell as in claim 50 wherein said cell is selected from the group consisting of fibroblast, liver, kidney, spleen, bone marrow and neurological cells.

54. A host cell as in claim 50 wherein said cell is an invertebrate cell.

55. A non-human animal model for cancer, wherein a genome of said animal, or an ancestor thereof, has been modified by at least one recombinant construct, and wherein said recombinant construct has introduced a modification selected from the group consisting of

(a) insertion of nucleotide sequences encoding at least a functional domain of a heterospecific normal CalDAG-GEF gene;

(b) insertion of nucleotide sequences encoding at least a functional domain of a heterospecific mutant CalDAG-GEF gene;

(c) insertion of nucleotide sequences encoding at least a functional domain of a conspecific homologue of a heterospecific mutant CalDAG-GEF gene;

(d) inactivation of an endogenous CalDAG-GEF gene;

(e) insertion of nucleotide sequences encoding at least a functional domain of a heterospecific normal cAMP-GEF gene;

(f) insertion of nucleotide sequences encoding at least a functional domain of a heterospecific mutant cAMP-GEF gene;

(g) insertion of nucleotide sequences encoding at least a functional domain of a conspecific homologue of a heterospecific mutant cAMP-GEF gene; and

(h) inactivation of an endogenous cAMP-GEF gene.

56. A non-human animal model as in claim 55 wherein said cancer is related to the Ras-pathway.

57. A non human animal model as in claim 56 wherein said cancer is selected from the group consisting of lung cancer, pancreatic cancer, breast cancer, colorectal cancer, and myeloid leukemia.

58. An animal model as in claim 55 wherein said modification is an insertion of a nucleotide sequence encoding at least a functional domain of a protein selected from the group consisting of a normal human CalDAG-GEF, and a normal cAMP-GEF gene.

59. An animal model as in claim 55 wherein said modification is an insertion of a nucleotide sequence encoding at least a functional domain of a protein selected from the group consisting of a mutant human CalDAG-GEF, and a mutant human cAMP-GEF gene.

60. An animal as in claim 55 wherein said animal is selected from the group consisting of rats, mice, hamsters, guinea pigs, rabbits, dogs, cats, goats, sheep, pigs, and non-human primates.

61. An animal as in claim 55 wherein said animal is an invertebrate.

62. A method for producing at least a functional domain of a protein selected from the group consisting of a CalDAG-GEF protein, and a cAMP-GEF protein, said method comprising culturing a host cell of any of claims 50-54 under suitable conditions to produce said protein by expressing said nucleic acid.

63. A substantially pure preparation of a protein selected from the group consisting of a normal CalDAG-GEF protein, a mutant CalDAG-GEF protein, a normal cAMP-GEF protein, and a mutant cAMP-GEF protein.

64. A substantially pure preparation as in claim 63 wherein said protein comprises a normal protein selected from the group consisting of

(a) a protein comprising the amino acid sequence of SEQ ID NO: 2;

(b) a protein comprising the amino acid sequence of SEQ ID NO: 4;

(c) a protein comprising the amino acid sequence of SEQ ID NO: 6;

(d) a protein comprising the amino acid sequence of SEQ ID NO: 8;

(e) a protein comprising the amino acid sequence of SEQ ID NO: 10;

(f) a protein comprising the amino acid sequence of SEQ ID NO: 12;

(g) a protein comprising the amino acid sequence of SEQ ID NO: 14;

(h) a protein comprising the amino acid sequence of SEQ ID NO: 16; and

(i) a protein comprising the amino acid sequence of SEQ ID NO: 18.

65. A substantially pure preparation of a polypeptide comprising an amino acid sequence of at least 4 consecutive amino acid residues selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18.

66. A substantially pure preparation of a polypeptide comprising an amino acid sequence of at least 10 consecutive amino acid residues selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18.

67. A substantially pure preparation of a polypeptide comprising an amino acid sequence of at least 15 consecutive amino acid residues selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, and SEQ ID NO: 18.

68. A substantially pure preparation of a polypeptide comprising at least one functional domain of a protein selected from the group consisting of a normal CalDAG-GEF protein, a mutant CalDAG-GEF protein, a normal cAMP-GEF protein, and a mutant cAMP-GEF protein.

69. A substantially pure preparation of a polypeptide comprising an antigenic determinant of a protein selected from the group consisting of a normal CalDAG-GEF protein, a mutant CalDAG-GEF protein, a normal cAMP-GEF protein, and a mutant cAMP-GEF protein.

70. A method of producing antibodies which selectively bind to a CalDAG-GEF protein comprising the steps of

administering an immunogenically effective amount of a CalDAG-GEF immunogen to an animal;

allowing said animal to produce antibodies to said immunogen; and

obtaining said antibodies from said animal or from a cell culture derived therefrom.

71. A method of producing antibodies which selectively bind to a cAMP-GEF protein comprising the steps of

administering an immunogenically effective amount of a cAMP-GEF immunogen to an animal;

allowing said animal to produce antibodies to said immunogen; and

obtaining said antibodies from said animal or from a cell culture derived therefrom.

72. A substantially pure preparation of an antibody which selectively binds to an antigenic determinant of a protein selected from the group consisting of a normal CalDAG-GEF protein, a mutant CalDAG-GEF protein, a normal cAMP-GEF protein, and a mutant cAMP-GEF protein.

73. A substantially pure preparation of an antibody as in claim 72 wherein said antibody selectively binds to an antigenic determinant of a mutant CalDAG-GEF and fails to bind to a normal CalDAG-GEF protein.

74. A substantially pure preparation of an antibody as in claim 72 wherein said antibody selectively binds to an antigenic determinant of a mutant cAMP-GEF and fails to bind to a normal cAMP-GEF protein.

75. A cell line producing an antibody of any one of claims 72-74.

76. A method for identifying compounds which can modulate the expression of a CalDAG-GEF gene comprising:

contacting a cell with a test candidate wherein said cell includes a regulatory region of a CalDAG-GEF gene operably joined to a coding region; and

detecting a change in expression of said coding region.

77. A method for identifying compounds which can modulate the expression of a cAMP-GEF gene comprising:

contacting a cell with a test candidate wherein said cell includes a regulatory region of a cAMP-GEF gene operably joined to a coding region; and

detecting a change in expression of said coding region.

78. A method as in claim 76 or 77 wherein said change comprises a change in a level of an mRNA transcript encoded by said coding region.

79. A method as in claim 78 wherein said change comprises a change in a level of a protein encoded by said coding region.

80. A method as in claim 78 wherein said change is a result of an activity of a protein encoded by said coding region.

81. A method as in claim 78 wherein said coding region encodes a marker protein selected from the group consisting of β-galactosidase, alkaline phosphatase, green fluorescent protein, and luciferase.

82. A method for identifying compounds which can selectively bind to a CalDAG-GEF protein comprising the steps of

providing a preparation including at least one CalDAG-GEF component;

contacting said preparation with a sample including at least one candidate compound; and

detecting binding of said CalDAG-GEF component to said candidate compound.

83. A method for identifying compounds which can selectively bind to a cAMP-GEF protein comprising the steps of

providing a preparation including at least one cAMP-GEF component;

contacting said preparation with a sample including at least one candidate compound; and

detecting binding of said cAMP-GEF component to said candidate compound.

84. The method in claim 82 wherein said binding to said CalDAG-GEF component is detected by an assay selected from the group consisting of: affinity chromatography, co-immunoprecipitation, a Biomolecular Interaction Assay, and a yeast two-hybrid system.

85. The method in claim 83 wherein said binding to said cAMP-GEF component is detected by an assay selected from the group consisting of: affinity chromatography, co-immunoprecipitation, a Biomolecular Interaction Assay, and a yeast two-hybrid system.

86. A method of identifying compounds which can modulate activity of a CalDAG-GEF comprising the steps of

providing a cell expressing a normal or mutant CalDAG-GEF gene;

contacting said cell with at least one candidate compound; and

detecting a change in a marker of said activity.

87. A method of identifying compounds which can modulate activity of a cAMP-GEF comprising the steps of

providing a cell expressing a normal or mutant cAMP-GEF gene;

contacting said cell with at least one candidate compound; and

detecting a change in a marker of said activity.

88. A method as in claim 86 wherein measurement of said marker indicates a difference between cells bearing an expressed mutant CalDAG-GEF gene and otherwise identical cells free of an expressed mutant CalDAG-GEF gene.

89. A method as in claim 86 wherein said change comprises a change in a non-specific marker of cell physiology selected from the group consisting of pH, intracellular calcium, cyclic AMP levels, GTP/GDP ratios, phosphatidylinositol activity, and protein phosphorylation.

90. A method as in claim 86 wherein said change comprises a change in expression of said CalDAG-GEF.

91. A method as in claim 86 wherein said change comprises a change in occurrence or rate of apoptosis or cell death.

92. A method as in claim 86 wherein said cell is a cell cultured *in vitro*.

93. A method as in claim 92 wherein said cell is a transformed host cell of any one of claims 50-54.

94. A method as in claim 92 wherein said cell is explanted from a host bearing at least one mutant CalDAG-GEF gene.

95. A method as in claim 92 wherein said cell is explanted from a transgenic animal of any one of claims 55-61.

96. A method as in claim 86 wherein said cell is a cell in a live animal.

97. A method as in claim 96 wherein said cell is a cell of a transgenic animal of any one of claims 55-61.

98. A method as in claim 86 wherein said cell is in a human subject in a clinical trial.

99. A method as in claim 87 wherein measurement of said marker indicates a difference between cells bearing an expressed mutant cAMP-GEF gene and otherwise identical cells free of an expressed mutant cAMP-GEF gene.

100. A method as in claim 87 wherein said change comprises a change in a non-specific marker of cell physiology selected from the group consisting of pH, intracellular calcium, cyclic AMP levels, GTP/GDP ratios, phosphatidylinositol activity, and protein phosphorylation.

101. A method as in claim 87 wherein said change comprises a change in expression of said cAMP-GEF.

102. A method as in claim 87 wherein said change comprises a change in occurrence or rate of apoptosis or cell death.

103. A method as in claim 87 wherein said cell is a cell cultured *in vitro*.

104. A method as in claim 103 wherein said cell is a transformed host cell of any one of claims 50-54.

105. A method as in claim 103 wherein said cell is explanted from a host bearing at least one mutant cAMP-GEF gene.

106. A method as in claim 103 wherein said cell is explanted from a transgenic animal of any one of claims 55-61.

107. A method as in claim 87 wherein said cell is a cell in a live animal.

108. A method as in claim 107 wherein said cell is a cell of a transgenic animal of any one of claims 55-61.

109. A method as in claim 87 wherein said cell is in a human subject in a clinical trial.

110. A diagnostic method for determining if a subject bears a mutant CalDAG-GEF gene comprising the steps of

providing a biological sample of said subject; and

detecting in said sample a mutant CalDAG-GEF nucleic acid, a mutant CalDAG-GEF protein, or a mutant CalDAG-GEF activity.

111. A method as in claim 111, wherein a mutant CalDAG-GEF nucleic acid is detected by an assay selected from the group consisting of direct nucleotide sequencing, probe specific hybridization, restriction enzyme digest and mapping, PCR mapping, ligase-mediated PCR detection, RNase protection, electrophoretic mobility shift detection, and chemical mismatch cleavage.

112. A method as in claim 110, wherein a mutant CalDAG-GEF protein is detected by an assay selected from the group consisting of an immunoassay, a protease assay, and an electrophoretic mobility assay.

113. A diagnostic method for determining if a subject bears a mutant cAMP-GEF gene comprising the steps of

providing a biological sample of said subject; and

detecting in said sample a mutant cAMP-GEF nucleic acid, a mutant cAMP-GEF protein, or a mutant cAMP-GEF activity.

114. A method as in claim 113, wherein a mutant cAMP-GEF nucleic acid is detected by an assay selected from the group consisting of direct nucleotide sequencing, probe specific hybridization, restriction enzyme digest and mapping, PCR mapping, ligase-mediated PCR detection, RNase protection, electrophoretic mobility shift detection, and chemical mismatch cleavage.

115. A method as in claim 113, wherein a mutant cAMP-GEF protein is detected by an assay selected from the group consisting of an immunoassay, a protease assay, and an electrophoretic mobility assay.

116. A pharmaceutical preparation comprising a substantially pure CalDAG-GEF protein and a pharmaceutically acceptable carrier.

117. A pharmaceutical preparation comprising a substantially pure cAMP-GEF protein and a pharmaceutically acceptable carrier.

118. A pharmaceutical preparation comprising an expression vector operably encoding a protein selected from the group consisting of a CalDAG-GEF protein, and a cAMP-GEF protein, wherein said expression vector may express said CalDAG-GEF protein or said cAMP-GEF protein in a human subject, and a pharmaceutically acceptable carrier.

119. A pharmaceutical preparation comprising an expression vector operably encoding a CalDAG-GEF antisense sequence, wherein said expression vector may express said CalDAG-GEF antisense sequence in a human subject, and a pharmaceutically acceptable carrier.

120. A pharmaceutical preparation comprising an expression vector operably encoding a cAMP-GEF antisense sequence, wherein said expression vector may express said cAMP-GEF antisense sequence in a human subject, and a pharmaceutically acceptable carrier.

121. A pharmaceutical preparation comprising a substantially pure antibody, and a pharmaceutically acceptable carrier,

wherein said antibody selectively binds to a mutant protein selected from the group consisting of a mutant CalDAG-GEF protein, and a mutant cAMP-GEF protein.

122. A pharmaceutical preparation as in claim 121 wherein said preparation is essentially free of an antibody which selectively binds a normal CalDAG-GEF protein.

123. A pharmaceutical preparation as in claim 121 wherein said preparation is essentially free of an antibody which selectively binds a normal cAMP-GEF protein.

124. A pharmaceutical preparation comprising a substantially pure preparation of an antigenic determinant of a mutant CalDAG-GEF protein or a mutant cAMP-GEF protein.

125. A pharmaceutical preparation as in claim 124 wherein said preparation is essentially free of an antigenic determinant of a normal CalDAG-GEF protein.

126. A pharmaceutical preparation as in claim 124 wherein said preparation is essentially free of an antigenic determinant of a normal cAMP-GEF protein.

127. A method of treatment for a patient bearing a mutant CalDAG-GEF gene comprising the step of administering to said patient a therapeutically effective amount of the pharmaceutical preparation of claim 116.

128. A method for identifying compounds according to claim 82, wherein the CalDAG-GEF component is a CalDAG-GEF domain selected from the group consisting of SCR1, SCR2, SCR3, DAG-binding and an EF hand domain.

129. A substantially pure preparation of a polypeptide comprising a domain selected from the group consisting of a CalDAG-GEF SCR1 domain, a CalDAG-GEF SCR2 domain, CalDAG-GEF SCR3 domain, CalDAG-GEF DAG-binding domain, CalDAG-GEF EF hand domain.

130. A substantially pure preparation of a polypeptide comprising a domain selected from the group consisting of a cAMP-GEF SCR1 domain, a cAMP-GEF SCR2 domain, cAMP-GEF SCR3 domain, cAMP-GEF cAMP-binding domain.

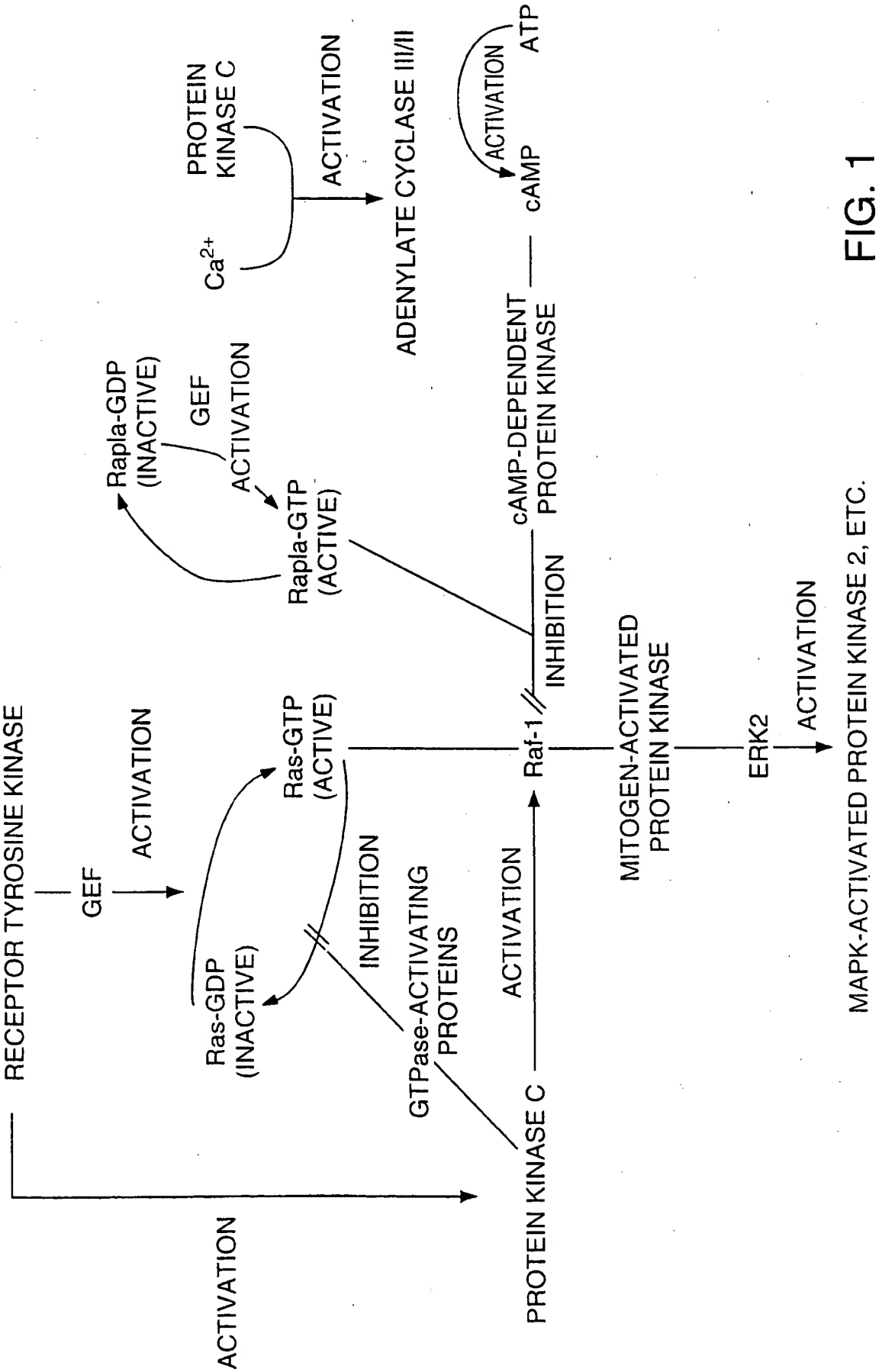

RECEPTOR TYROSINE KINASE
GEF
ACTIVATION
Ras-GDP (INACTIVE)
Ras-GTP (ACTIVE)
INHIBITION
GTPase-ACTIVATING PROTEINS
ACTIVATION
PROTEIN KINASE C
ACTIVATION
Raf-1
INHIBITION
MITOGEN-ACTIVATED PROTEIN KINASE
ERK2
ACTIVATION
MAPK-ACTIVATED PROTEIN KINASE 2, ETC.
Rapla-GDP (INACTIVE)
GEF ACTIVATION
Rapla-GTP (ACTIVE)
cAMP-DEPENDENT PROTEIN KINASE
ADENYLATE CYCLASE III/II
ACTIVATION
ATP
cAMP
PROTEIN KINASE C
Ca^{2+}
ACTIVATION

FIG. 1

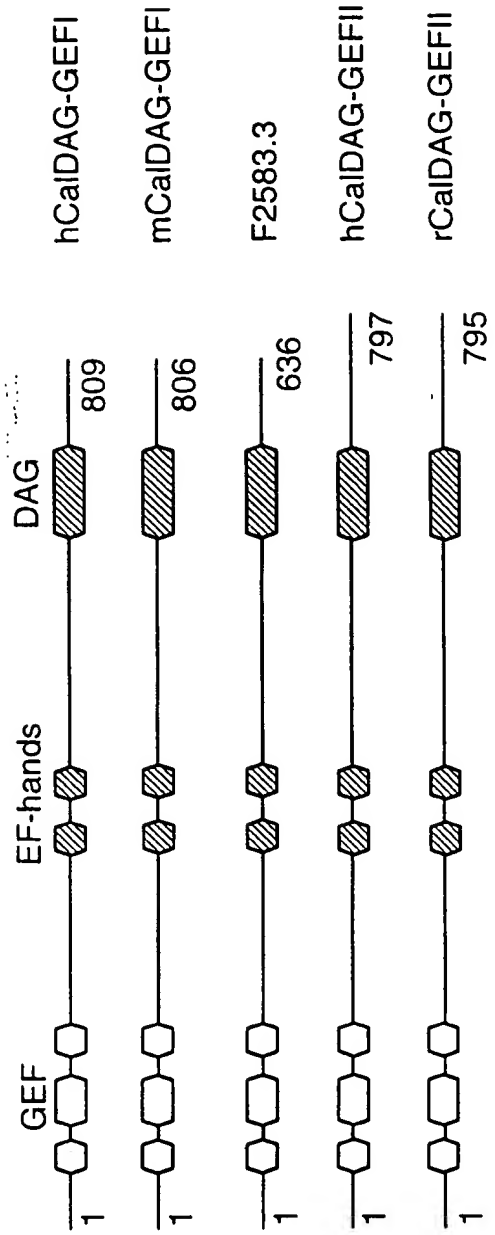

GEF
EF-hands
DAG
hCalDAG-GEFI
mCalDAG-GEFI
F2583.3
hCalDAG-GEFII
rCalDAG-GEFII
1
809
806
636
797
795

FIG. 2A

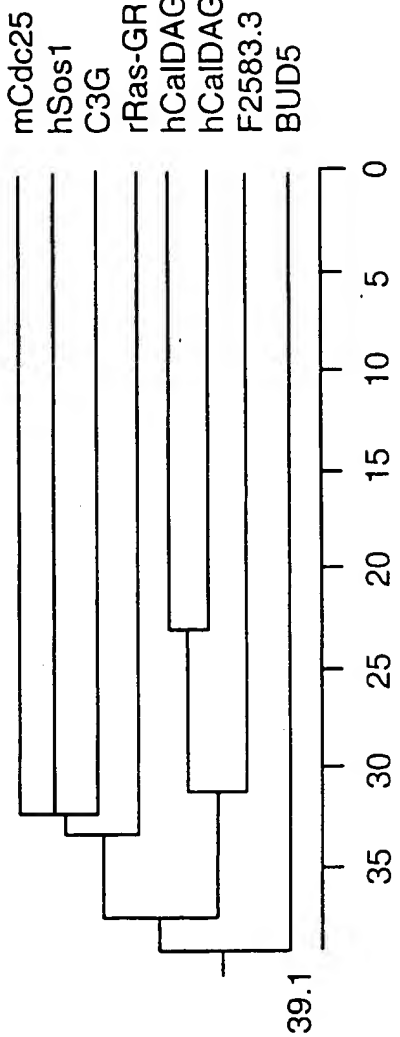

mCdc25
hSos1
C3G
rRas-GRF
hCalDAG-GEFI
hCalDAG-GEFII
F2583.3
BUD5
39.1
35
30
25
20
15
10
5
0

FIG. 2B

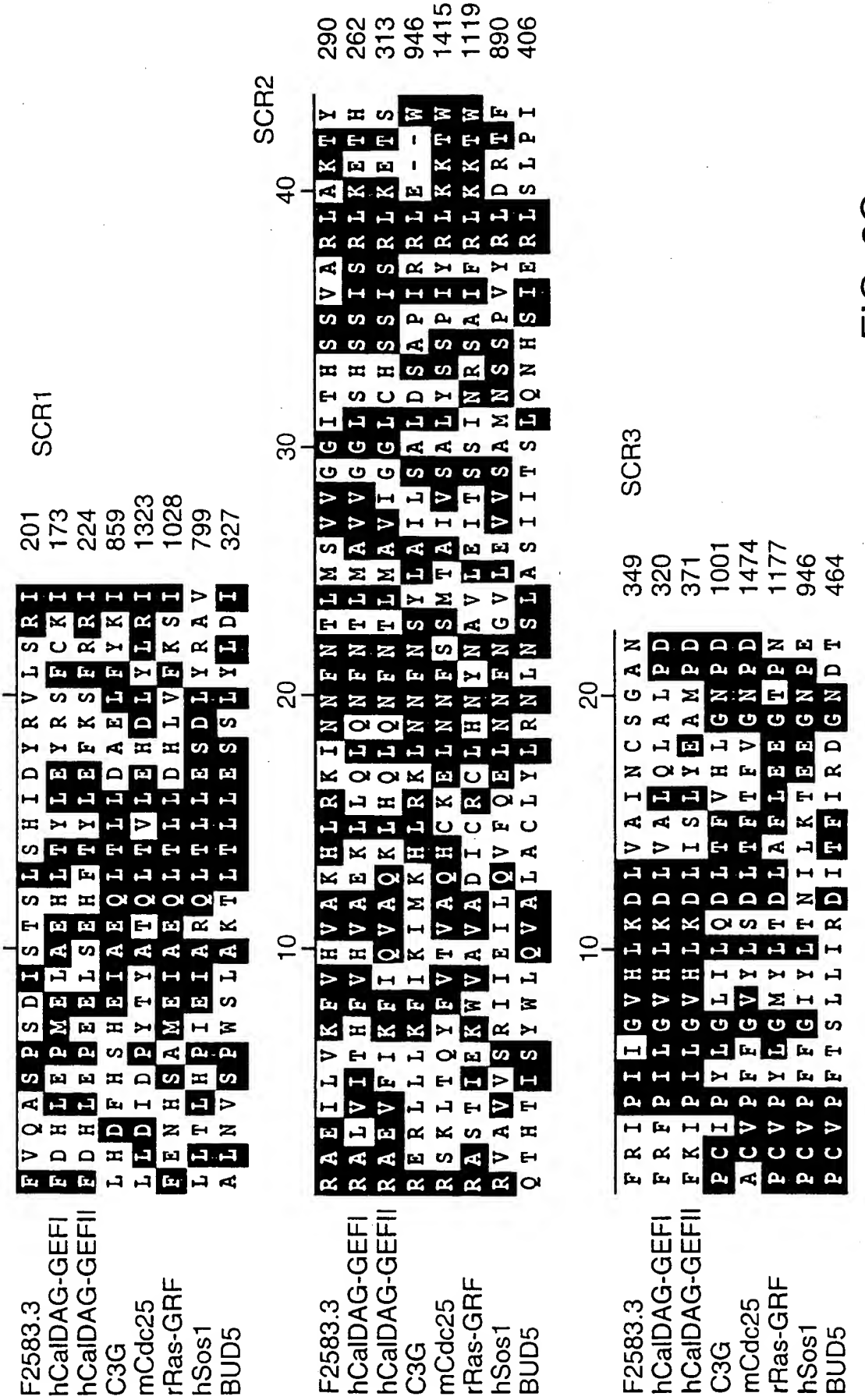

SCR1
SCR2
SCR3
F2583.3
hCalDAG-GEFI
hCalDAG-GEFII
C3G
mCdc25
rRas-GRF
hSos1
BUD5
201
173
224
859
1323
1028
799
327
290
262
313
946
1415
1119
890
406
349
320
371
1001
1474
1177
946
464

FIG. 2C

FIG. 2D

FIG. 2D-1	FIG. 2D-2

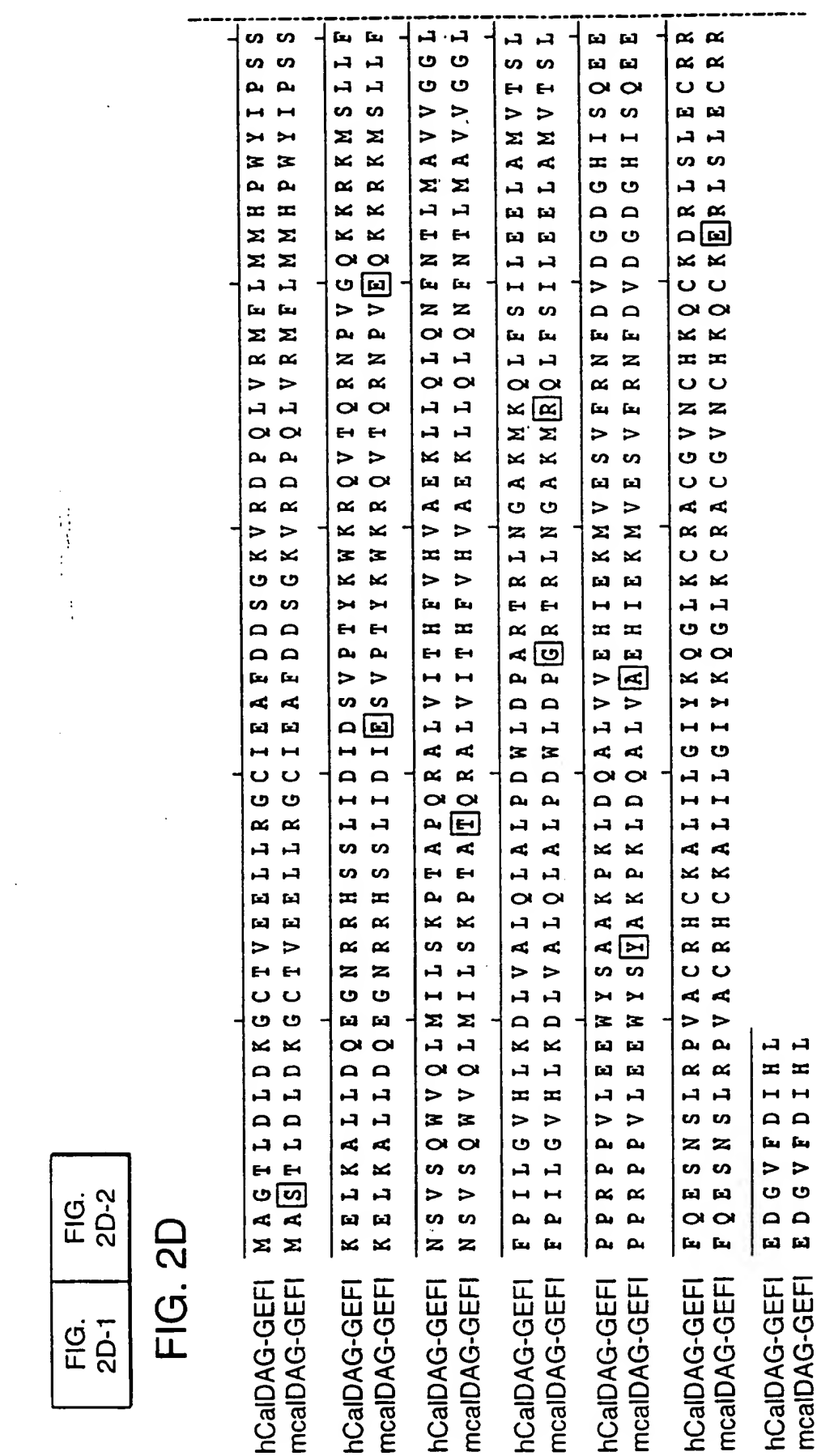


FIG. 2D-1

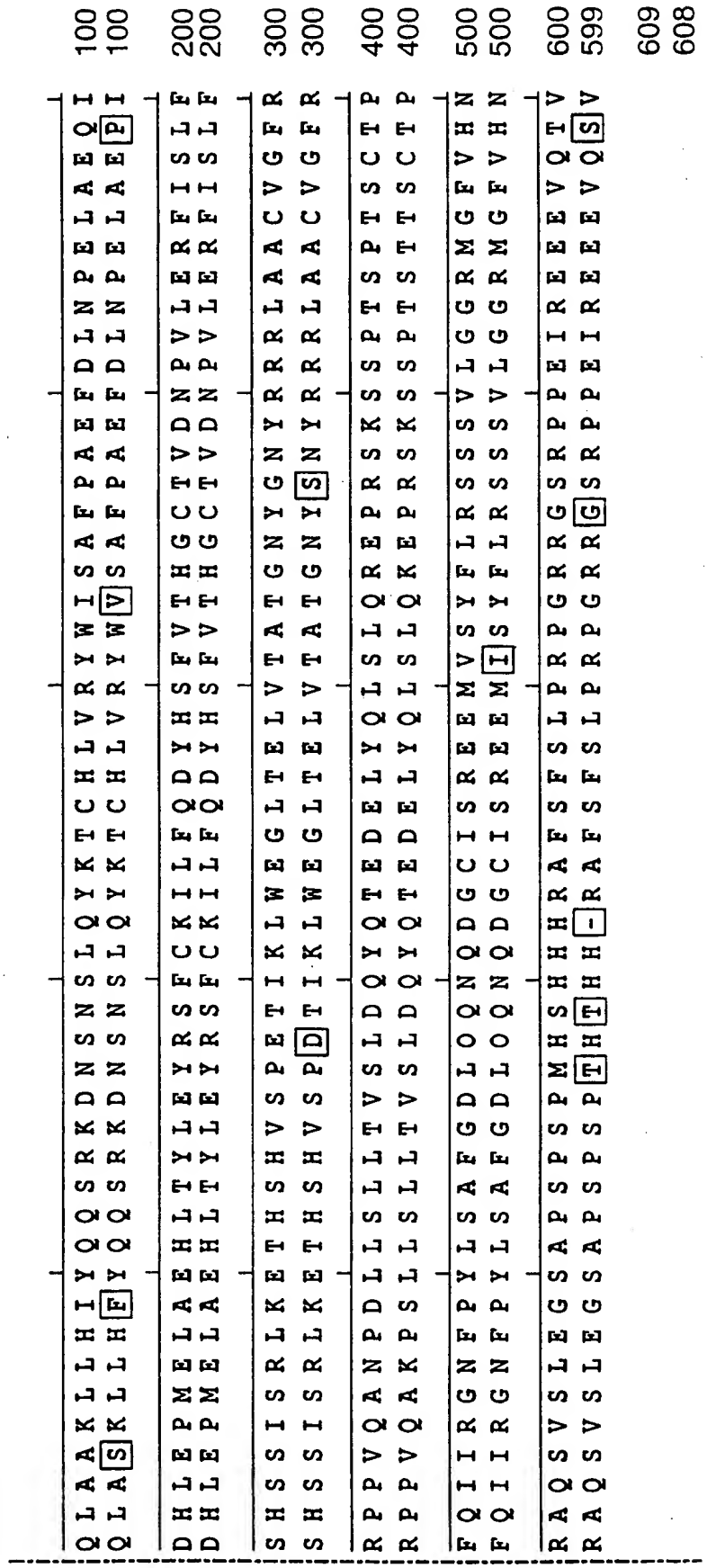


FIG. 2D-2

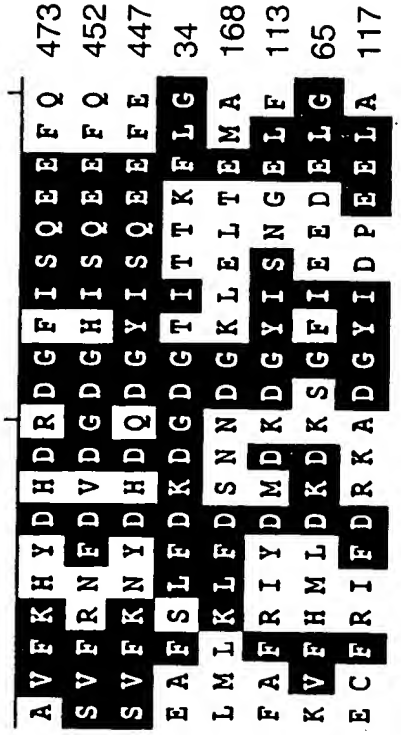

F2583.3
hCalDAG-GEFI
hCalDAG-GEFII
hCalmodulin
hCalbindin D28K
hCalcineurin B
hParvalbumin α
hTroponin C
20
30
473
452
447
34
168
113
65
117

FIG. 2E

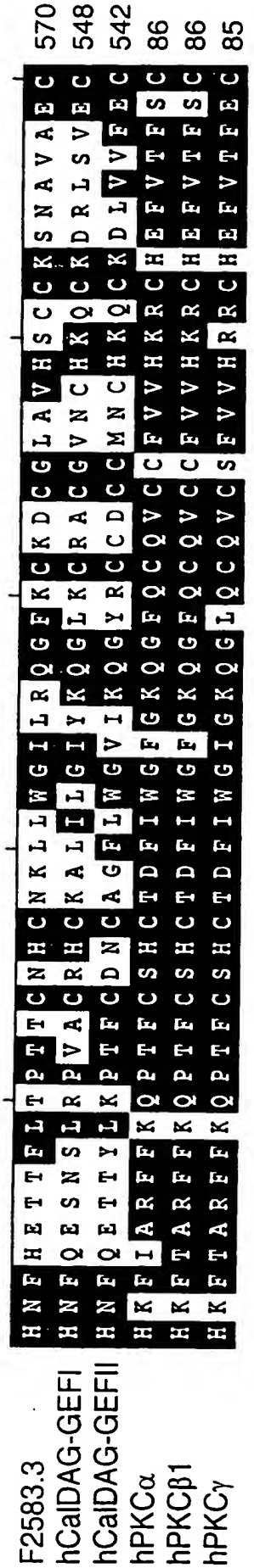

F2583.3
hCalDAG-GEFI
hCalDAG-GEFII
hPKCα
hPKCβ1
hPKCγ
10
20
30
40
50
570
548
542
86
86
85

FIG. 2F

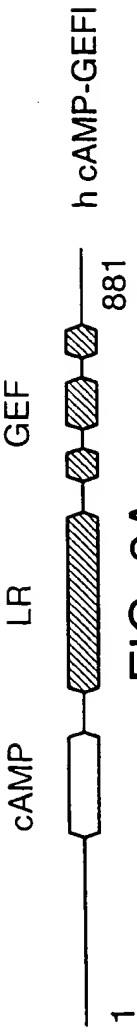


FIG. 3A

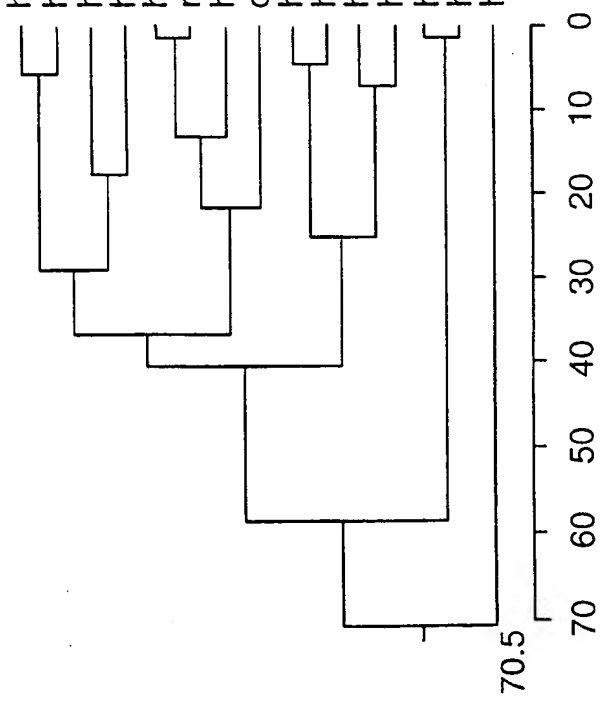


FIG. 3B

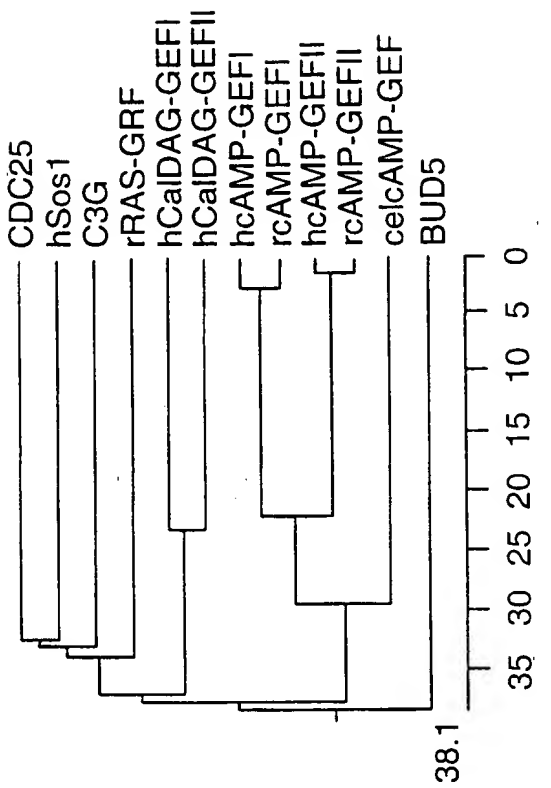
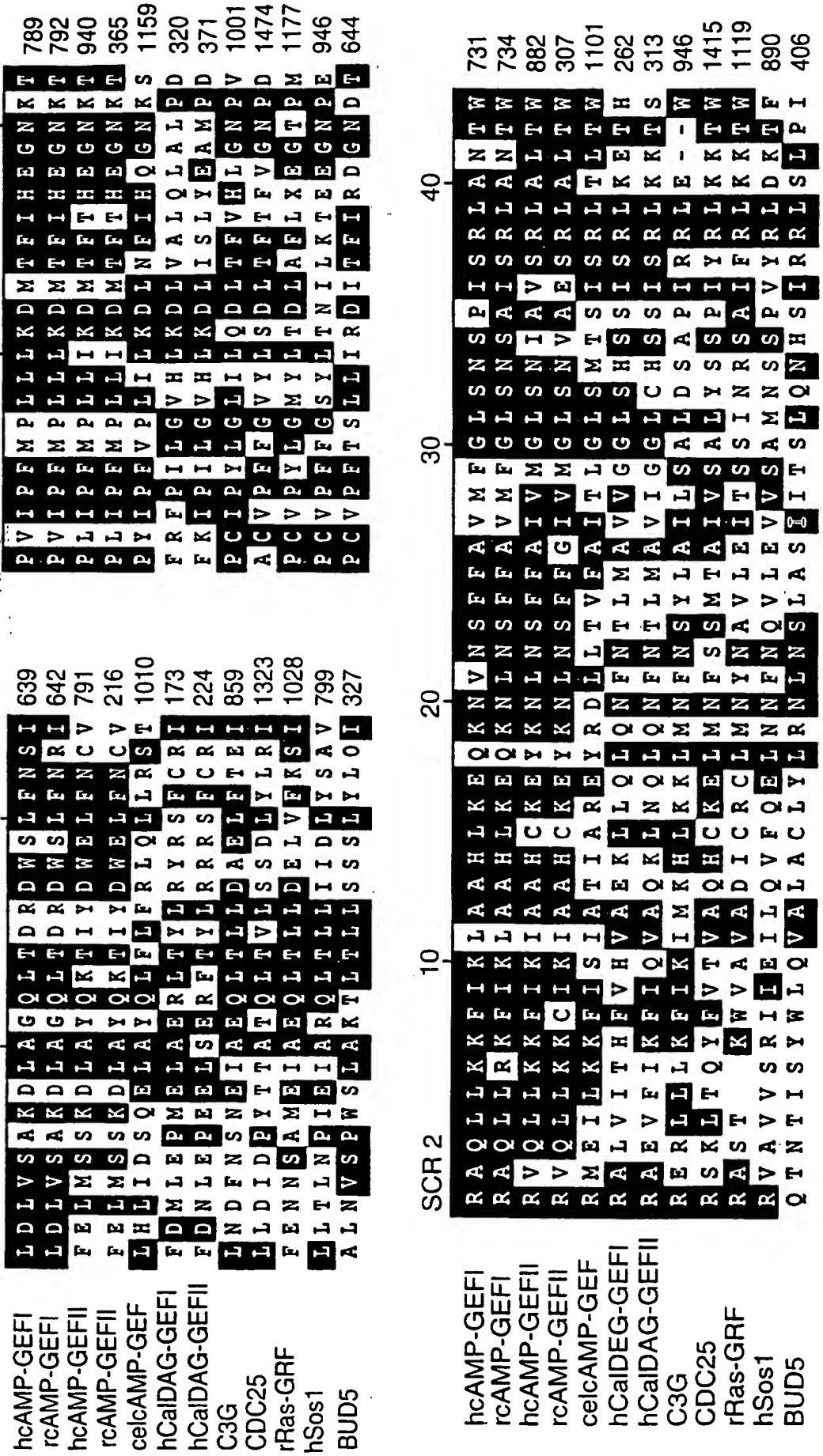


FIG. 3C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SCR 2
SCR 3
hcAMP-GEFI
rcAMP-GEFI
hcAMP-GEFII
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CDC25
rRas-GRF
hSos1
BUD5

FIG. 3D

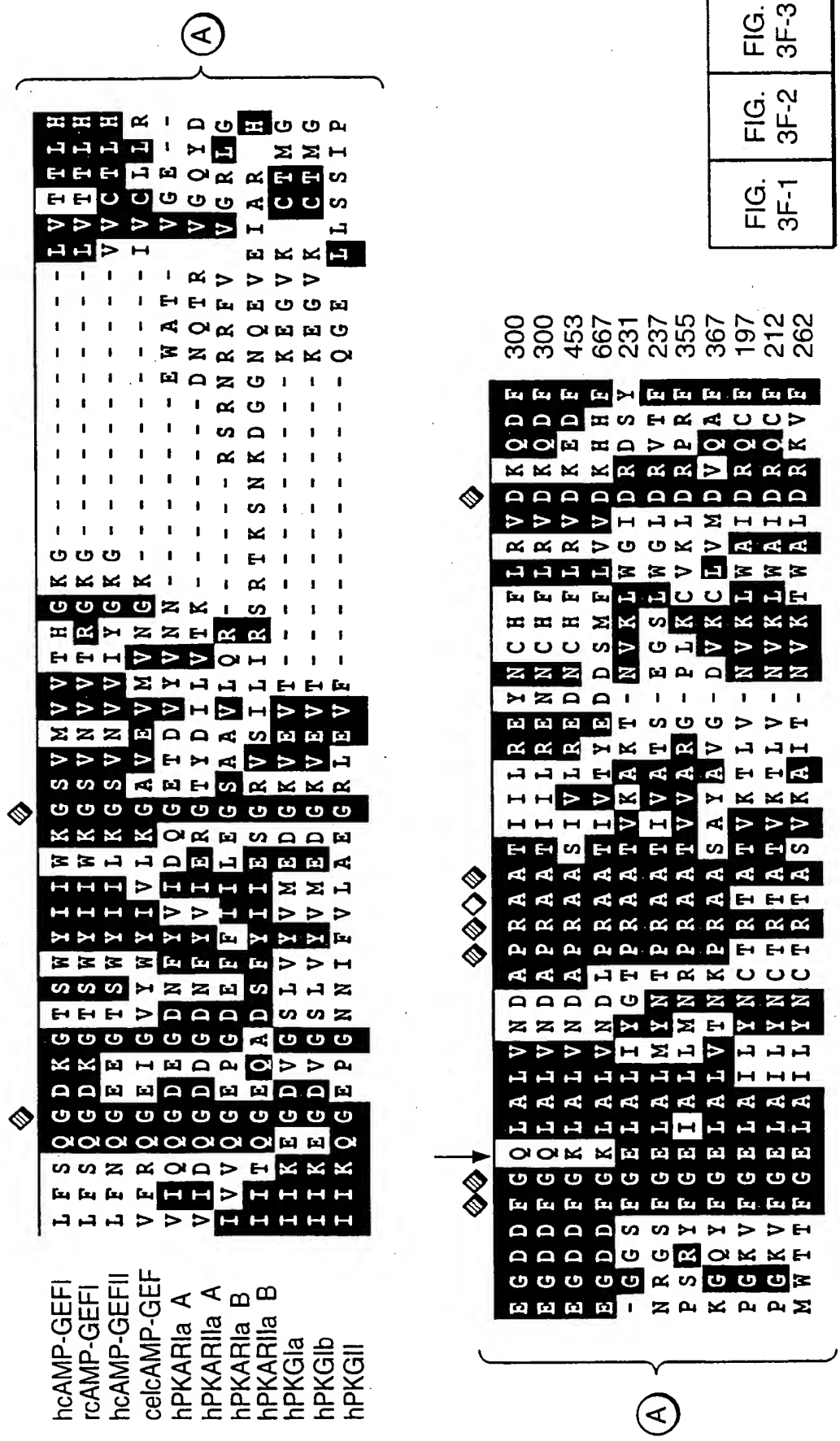
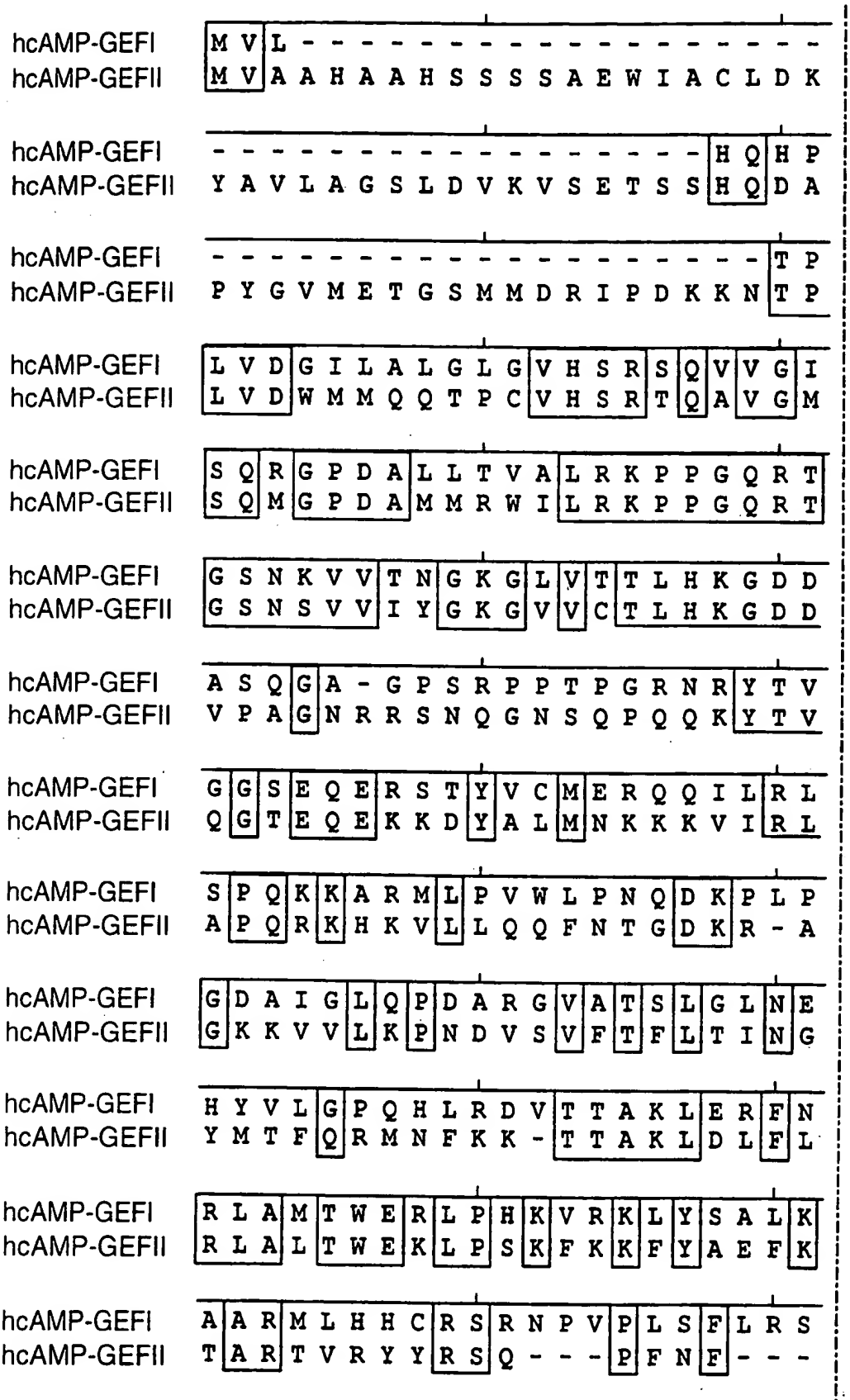


FIG. 3E

FIG. 3F-1 | FIG. 3F-2 | FIG. 3F-3

FIG. 3F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hcAMP-GEFII M V A A H A A H S S S S A E W I A C L D K
hcAMP-GEFI - - - - - - - - - - - - - - - - - - H Q H P
hcAMP-GEFII Y A V L A G S L D V K V S E T S S H Q D A
hcAMP-GEFI - - - - - - - - - - - - - - - - - - - T P
hcAMP-GEFII P Y G V M E T G S M M D R I P D K K N T P
hcAMP-GEFI L V D G I L A L G L G V H S R S Q V V G I
hcAMP-GEFII L V D W M M Q Q T P C V H S R T Q A V G M
hcAMP-GEFI S Q R G P D A L L T V A L R K P P G Q R T
hcAMP-GEFII S Q M G P D A M M R W I L R K P P G Q R T
hcAMP-GEFI G S N K V V T N G K G L V T T L H K G D D
hcAMP-GEFII G S N S V V I Y G K G V V C T L H K G D D
hcAMP-GEFI A S Q G A - G P S R P P T P G R N R Y T V
hcAMP-GEFII V P A G N R R S N Q G N S Q P Q Q K Y T V
hcAMP-GEFI G G S E Q E R S T Y V C M E R Q Q I L R L
hcAMP-GEFII Q G T E Q E K K D Y A L M N K K K V I R L
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hcAMP-GEFII A P Q R K H K V L L Q Q F N T G D K R - A
hcAMP-GEFI G D A I G L Q P D A R G V A T S L G L N E
hcAMP-GEFII G K K V V L K P N D V S V F T F L T I N G
hcAMP-GEFI H Y V L G P Q H L R D V T T A K L E R F N
hcAMP-GEFII Y M T F Q R M N F K K - T T A K L D L F L
hcAMP-GEFI R L A M T W E R L P H K V R K L Y S A L K
hcAMP-GEFII R L A L T W E K L P S K F K K F Y A E F K
hcAMP-GEFI A A R M L H H C R S R N P V P L S F L R S
hcAMP-GEFII T A R T V R Y Y R S Q - - - P F N F - - -

FIG. 3F-1

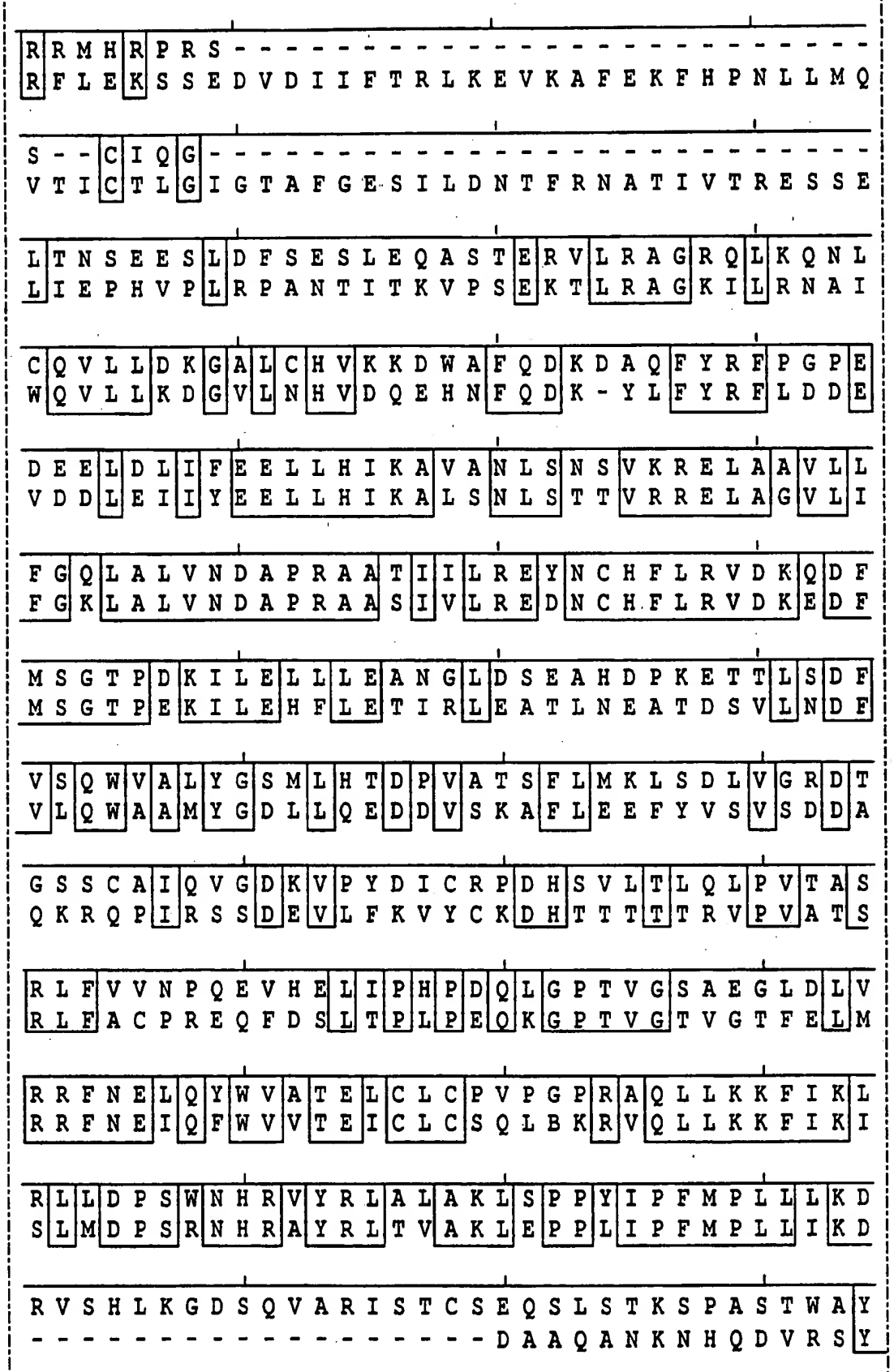
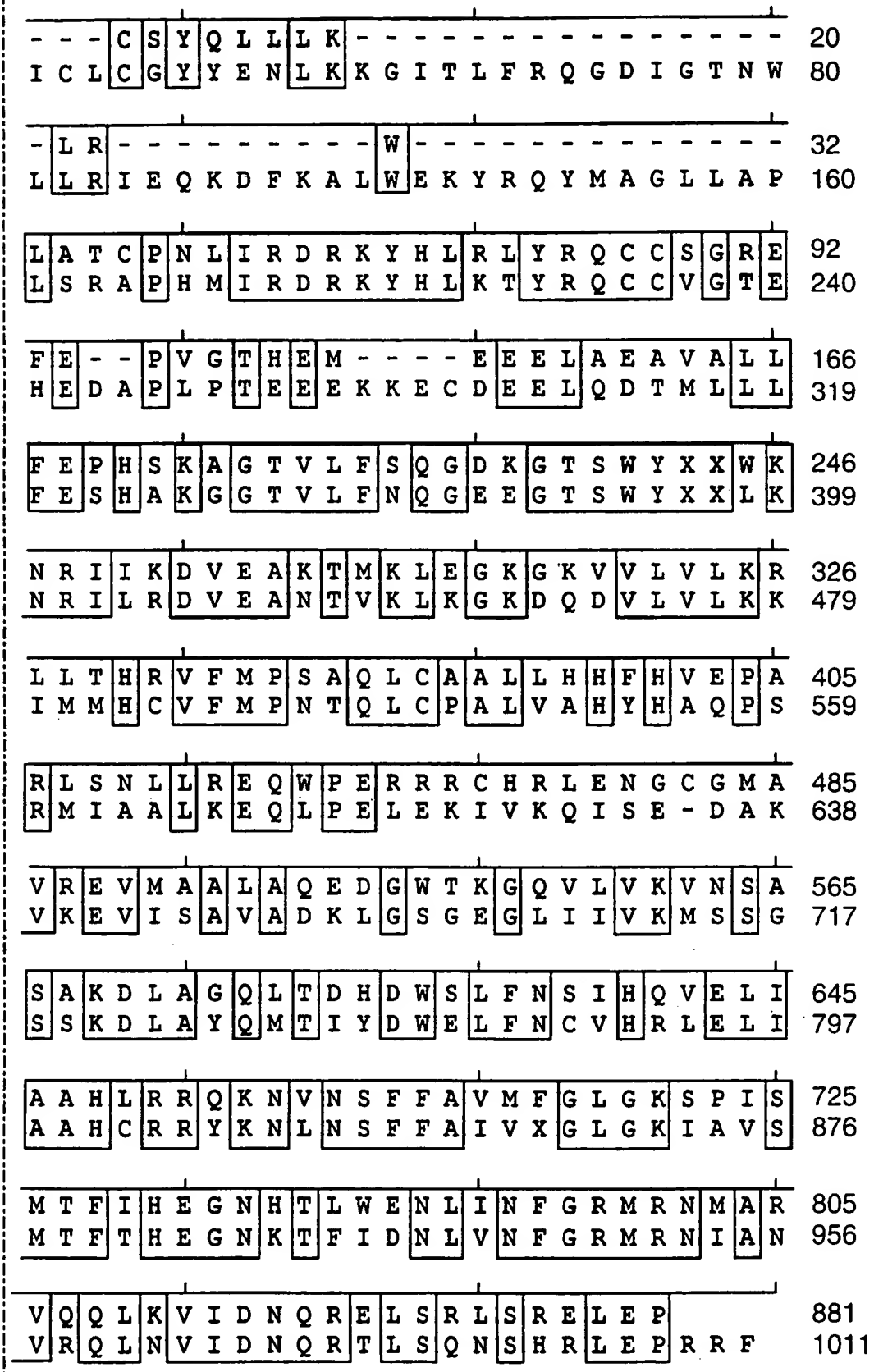


FIG. 3F-2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I C L C G Y Y E N L K K G I T L F R Q G D I G T N W 80
- L R - - - - - - - - - - W - - - - - - - - - - - - - 32
L L R I E Q K D F K A L W E K Y R Q Y M A G L L A P 160
L A T C P N L I R D R K Y H L R L Y R Q C C S G R E 92
L S R A P H M I R D R K Y H L K T Y R Q C C V G T E 240
F E - - P V G T H E M - - - - E E E L A E A V A L L 166
H E D A P L P T E E E K K E C D E E L Q D T M L L L 319
F E P H S K A G T V L F S Q G D K G T S W Y X X W K 246
F E S H A K G G T V L F N Q G E E G T S W Y X X L K 399
N R I I K D V E A K T M K L E G K G K V V L V L K R 326
N R I L R D V E A N T V K L K G K D Q D V L V L K K 479
L L T H R V F M P S A Q L C A A L L H H F H V E P A 405
I M M H C V F M P N T Q L C P A L V A H Y H A Q P S 559
R L S N L L R E Q W P E R R R C H R L E N G C G M A 485
R M I A A L K E Q L P E L E K I V K Q I S E - D A K 638
V R E V M A A L A Q E D G W T K G Q V L V K V N S A 565
V K E V I S A V A D K L G S G E G L I I V K M S S G 717
S A K D L A G Q L T D H D W S L F N S I H Q V E L I 645
S S K D L A Y Q M T I Y D W E L F N C V H R L E L I 797
A A H L R R Q K N V N S F F A V M F G L G K S P I S 725
A A H C R R Y K N L N S F F A I V X G L G K I A V S 876
M T F I H E G N H T L W E N L I N F G R M R N M A R 805
M T F T H E G N K T F I D N L V N F G R M R N I A N 956
V Q Q L K V I D N Q R E L S R L S R E L E P 881
V R Q L N V I D N Q R T L S Q N S H R L E P R R F 1011

FIG. 3F-3

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<110> Kawasaki, Hiroaki
Graybiel, Ann
Housman, David

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gctctgagag tgtaacttgg gtctagccca ctggcaccgg cagcc atg gcg agc act 177
Met Ala Ser Thr
1

ctg gac ctg gac aag ggt tgc acc gtg gag gag ctg ctc cgt ggc tgt 225
Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu Leu Arg Gly Cys
5 10 15 20

atc gaa gcc ttt gat gac tct gga aag gtg cga gat cca cag cta gtg 273
Ile Glu Ala Phe Asp Asp Ser Gly Lys Val Arg Asp Pro Gln Leu Val
25 30 35

cgc atg ttt ctc atg atg cac ccc tgg tac ata cct tcc tct cag ctg 321
Arg Met Phe Leu Met Met His Pro Trp Tyr Ile Pro Ser Ser Gln Leu
40 45 50

gct tcg aaa ctg ctc cac ttc tat cag caa tcc cgg aag gac aac tcc 369
Ala Ser Lys Leu Leu His Phe Tyr Gln Gln Ser Arg Lys Asp Asn Ser
55 60 65

aat tcc cta cag gtg aaa acc tgt cac ctg gtc agg tac tgg gtc tca 417
Asn Ser Leu Gln Val Lys Thr Cys His Leu Val Arg Tyr Trp Val Ser
70 75 80

gcc ttc cca gca gag ttc gac ttg aac cca gag ctg gct gaa ccg atc 465
Ala Phe Pro Ala Glu Phe Asp Leu Asn Pro Glu Leu Ala Glu Pro Ile
85 90 95 100

aag gag ctg aag gct ctg tta gac caa gaa gga aac cgc agg cac agc 513
Lys Glu Leu Lys Ala Leu Leu Asp Gln Glu Gly Asn Arg Arg His Ser
105 110 115

agc ctc atc gac atc gag agt gtc ccc acc tac aag tgg aag cgg cag 561
Ser Leu Ile Asp Ile Glu Ser Val Pro Thr Tyr Lys Trp Lys Arg Gln
120 125 130

gtg acc cag cgg aac cct gtg gaa cag aaa aag cgc aag atg tcc ctg 609
Val Thr Gln Arg Asn Pro Val Glu Gln Lys Lys Arg Lys Met Ser Leu
135 140 145

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ttg ttt gat cac ttg gag cct atg gaa ctg gca gaa cat ctc acc tac   657
Leu Phe Asp His Leu Glu Pro Met Glu Leu Ala Glu His Leu Thr Tyr
    150                 155                 160

ttg gag tat cgg tcc ttc tgc aag atc ctg ttc cag gac tat cac agc   705
Leu Glu Tyr Arg Ser Phe Cys Lys Ile Leu Phe Gln Asp Tyr His Ser
165                 170                 175                 180

ttt gtg act cat ggc tgc act gta gac aat ccg gtc ctg gag cga ttc   753
Phe Val Thr His Gly Cys Thr Val Asp Asn Pro Val Leu Glu Arg Phe
                185                 190                 195

atc tcc ctc ttc aac agt gtc tct cag tgg gtc caa ctc atg atc ctc   801
Ile Ser Leu Phe Asn Ser Val Ser Gln Trp Val Gln Leu Met Ile Leu
            200                 205                 210

agc aag ccc aca gcc acg cag cgg gcg ctg gtc atc aca cat ttc gtg   849
Ser Lys Pro Thr Ala Thr Gln Arg Ala Leu Val Ile Thr His Phe Val
        215                 220                 225

cat gtg gca gag aag ctg ctg cag ctg cag aac ttc aac acg ttg atg   897
His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe Asn Thr Leu Met
    230                 235                 240

gcc gtc gtg gga ggc ctg agc cac agc tcc atc tca cgc ctc aag gag   945
Ala Val Val Gly Gly Leu Ser His Ser Ser Ile Ser Arg Leu Lys Glu
245                 250                 255                 260

acc cac agc cat gtc agc cct gac acc atc aag ctc tgg gaa ggt ctg   993
Thr His Ser His Val Ser Pro Asp Thr Ile Lys Leu Trp Glu Gly Leu
                265                 270                 275

aca gaa cta gtg aca gct act ggc aac tac agc aac tac cgg cga agg   1041
Thr Glu Leu Val Thr Ala Thr Gly Asn Tyr Ser Asn Tyr Arg Arg Arg
            280                 285                 290

ctg gcg gcc tgc gtg ggc ttc cgc ttt cct atc ctg ggt gtg cac ctc   1089
Leu Ala Ala Cys Val Gly Phe Arg Phe Pro Ile Leu Gly Val His Leu
        295                 300                 305

aag gat cta gtg gct ctg cag ctg gct ctg cct gac tgg ctg gac cca   1137
Lys Asp Leu Val Ala Leu Gln Leu Ala Leu Pro Asp Trp Leu Asp Pro
    310                 315                 320

ggt cgg acc cgg ctc aat gga gcc aag atg agg cag ctt ttc agc att   1185
Gly Arg Thr Arg Leu Asn Gly Ala Lys Met Arg Gln Leu Phe Ser Ile
325                 330                 335                 340

ctg gag gag ttg gct atg gtg acc agt ctt cga cca cca gtg caa gcc   1233
Leu Glu Glu Leu Ala Met Val Thr Ser Leu Arg Pro Pro Val Gln Ala
                345                 350                 355

aac ccc gac ctg ctg agt ctg ctc acg gtg tcc ctg gac cag tat cag   1281
Asn Pro Asp Leu Leu Ser Leu Leu Thr Val Ser Leu Asp Gln Tyr Gln
            360                 365                 370

acg gag gat gag ctg tat cag ctc tct ctg cag cga gag cca cgt tcc   1329
Thr Glu Asp Glu Leu Tyr Gln Leu Ser Leu Gln Arg Glu Pro Arg Ser
        375                 380                 385

aag tca tcg ccc acc agc ccc acc agc tgc acc ccg cct ccc cgg ccg   1377
Lys Ser Ser Pro Thr Ser Pro Thr Ser Cys Thr Pro Pro Pro Arg Pro
    390                 395                 400

cct gtg ctg gaa gag tgg acc tca gtt gcc aag cct aag ctg gac caa   1425
Pro Val Leu Glu Glu Trp Thr Ser Val Ala Lys Pro Lys Leu Asp Gln
405                 410                 415                 420

gcc ttg gtg gcc gag cac att gag aag atg gtg gag tct gtg ttc cgg   1473
Ala Leu Val Ala Glu His Ile Glu Lys Met Val Glu Ser Val Phe Arg
                425                 430                 435

aac ttt gac gtt gat ggg gac ggt cac atc tcc cag gag gag ttc cag   1521
Asn Phe Asp Val Asp Gly Asp Gly His Ile Ser Gln Glu Glu Phe Gln
```

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            440                 445                 450

atc atc cgg ggc aac ttc cct tat ctc agc gcc ttt ggg gac ttg gac   1569
Ile Ile Arg Gly Asn Phe Pro Tyr Leu Ser Ala Phe Gly Asp Leu Asp
        455                 460                 465

cag aac cag gat ggc tgc atc agc cgg gag gag atg att tcc tac ttc   1617
Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met Ile Ser Tyr Phe
    470                 475                 480

ctg cgc tcc agc tcc gtg ctg gga ggc cgc atg ggc ttc gta cac aac   1665
Leu Arg Ser Ser Ser Val Leu Gly Gly Arg Met Gly Phe Val His Asn
485                 490                 495                 500

ttc cag gag agt aac tcg cta agg ccg gtc gcc tgc cga cac tgc aaa   1713
Phe Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys Arg His Cys Lys
                505                 510                 515

gct ctg atc ctg ggc atc tac aag cag ggc ctc aaa tgt aga gct tgt   1761
Ala Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys Cys Arg Ala Cys
            520                 525                 530

ggt gtg aac tgc cac aag cag tgc aaa gac cga ctg tca gtg gaa tgt   1809
Gly Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu Ser Val Glu Cys
        535                 540                 545

cgc cgc cgc gcc cag agt gtg agc ctg gag ggc tct gca ccc tct ccc   1857
Arg Arg Arg Ala Gln Ser Val Ser Leu Glu Gly Ser Ala Pro Ser Pro
    550                 555                 560

tca ccc aca cat acc cac cat cgg gcc ttc agc ttc tcc ctg cct cgc   1905
Ser Pro Thr His Thr His His Arg Ala Phe Ser Phe Ser Leu Pro Arg
565                 570                 575                 580

cca ggc agg cgc agc tct cgg cct cca gag atc cgt gaa gag gag gtg   1953
Pro Gly Arg Arg Ser Ser Arg Pro Pro Glu Ile Arg Glu Glu Glu Val
                585                 590                 595

cag act gtg gaa gat ggt gtg ttc gac atc cac tta taagacgctg        1999
Gln Thr Val Glu Asp Gly Val Phe Asp Ile His Leu
            600                 605

tgactatcaa ggactcattc ctgccttgga gaaaagactt ggagcagagc agggagccag 2059

ggattctggg gcaggaggtt ggggctgaag gtgggggaag ttgaaggtgg catgcactga 2119

aaaaaaggcc agggctggtg tccctaaggt tgtacagact tctgtgaata tttgtatttt 2179

ccagatggaa taaaaaggcc cgaataatta acctcgaaaa aaaaaaaaaa aaaaaaaaaa 2239

aaaaaaaaaa a                                                     2250


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<212> PRT
<213> Mus musculus

<400> 2
Met Ala Ser Thr Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu
  1               5                  10                  15

Leu Arg Gly Cys Ile Glu Ala Phe Asp Asp Ser Gly Lys Val Arg Asp
            20                  25                  30

Pro Gln Leu Val Arg Met Phe Leu Met Met His Pro Trp Tyr Ile Pro
        35                  40                  45

Ser Ser Gln Leu Ala Ser Lys Leu Leu His Phe Tyr Gln Gln Ser Arg
    50                  55                  60

Lys Asp Asn Ser Asn Ser Leu Gln Val Lys Thr Cys His Leu Val Arg
65                  70                  75                  80

Tyr Trp Val Ser Ala Phe Pro Ala Glu Phe Asp Leu Asn Pro Glu Leu
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85 90 95

Ala Glu Pro Ile Lys Glu Leu Lys Ala Leu Leu Asp Gln Glu Gly Asn
100 105 110

Arg Arg His Ser Ser Leu Ile Asp Ile Glu Ser Val Pro Thr Tyr Lys
115 120 125

Trp Lys Arg Gln Val Thr Gln Arg Asn Pro Val Glu Gln Lys Lys Arg
130 135 140

Lys Met Ser Leu Leu Phe Asp His Leu Glu Pro Met Glu Leu Ala Glu
145 150 155 160

His Leu Thr Tyr Leu Glu Tyr Arg Ser Phe Cys Lys Ile Leu Phe Gln
165 170 175

Asp Tyr His Ser Phe Val Thr His Gly Cys Thr Val Asp Asn Pro Val
180 185 190

Leu Glu Arg Phe Ile Ser Leu Phe Asn Ser Val Ser Gln Trp Val Gln
195 200 205

Leu Met Ile Leu Ser Lys Pro Thr Ala Thr Gln Arg Ala Leu Val Ile
210 215 220

Thr His Phe Val His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe
225 230 235 240

Asn Thr Leu Met Ala Val Val Gly Gly Leu Ser His Ser Ser Ile Ser
245 250 255

Arg Leu Lys Glu Thr His Ser His Val Ser Pro Asp Thr Ile Lys Leu
260 265 270

Trp Glu Gly Leu Thr Glu Leu Val Thr Ala Thr Gly Asn Tyr Ser Asn
275 280 285

Tyr Arg Arg Arg Leu Ala Ala Cys Val Gly Phe Arg Phe Pro Ile Leu
290 295 300

Gly Val His Leu Lys Asp Leu Val Ala Leu Gln Leu Ala Leu Pro Asp
305 310 315 320

Trp Leu Asp Pro Gly Arg Thr Arg Leu Asn Gly Ala Lys Met Arg Gln
325 330 335

Leu Phe Ser Ile Leu Glu Glu Leu Ala Met Val Thr Ser Leu Arg Pro
340 345 350

Pro Val Gln Ala Asn Pro Asp Leu Leu Ser Leu Leu Thr Val Ser Leu
355 360 365

Asp Gln Tyr Gln Thr Glu Asp Glu Leu Tyr Gln Leu Ser Leu Gln Arg
370 375 380

Glu Pro Arg Ser Lys Ser Ser Pro Thr Ser Pro Thr Ser Cys Thr Pro
385 390 395 400

Pro Pro Arg Pro Pro Val Leu Glu Glu Trp Thr Ser Val Ala Lys Pro
405 410 415

Lys Leu Asp Gln Ala Leu Val Ala Glu His Ile Glu Lys Met Val Glu
420 425 430

Ser Val Phe Arg Asn Phe Asp Val Asp Gly Asp Gly His Ile Ser Gln
435 440 445

Glu Glu Phe Gln Ile Ile Arg Gly Asn Phe Pro Tyr Leu Ser Ala Phe
450 455 460

Gly Asp Leu Asp Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met
465 470 475 480

Ile Ser Tyr Phe Leu Arg Ser Ser Ser Val Leu Gly Gly Arg Met Gly

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                485                 490                 495

Phe Val His Asn Phe Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys
            500                 505                 510

Arg His Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys
        515                 520                 525

Cys Arg Ala Cys Gly Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu
    530                 535                 540

Ser Val Glu Cys Arg Arg Arg Ala Gln Ser Val Ser Leu Glu Gly Ser
545                 550                 555                 560

Ala Pro Ser Pro Ser Pro Thr His Thr His His Arg Ala Phe Ser Phe
                565                 570                 575

Ser Leu Pro Arg Pro Gly Arg Arg Ser Ser Arg Pro Pro Glu Ile Arg
            580                 585                 590

Glu Glu Glu Val Gln Thr Val Glu Asp Gly Val Phe Asp Ile His Leu
        595                 600                 605


<210> 3
<211> 2236
<212> DNA
<213> Homo sapiens

<220>
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<222> (161)..(1987)
<223> CalDAG-GEFI

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ggggactcaa ggctggcctg gctcaagtga acagcacgtc caggaggcga cctcgtccgc 60

gggtttgcat tctggggtgg acgagctggg ggttcggtcc gagcccggtg ggaggctccc 120

ggagcgcagc ctgggcccag cccaccccgc gccggcggcc atg gca ggc acc ctg   175
                                              Met Ala Gly Thr Leu
                                                1               5

gac ctg gac aag ggc tgc acg gtg gag gag ctg ctc cgc ggg tgc atc   223
Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu Leu Arg Gly Cys Ile
                10                  15                  20

gaa gcc ttc gat gac tcc ggg aag gtg cgg gac ccg cag ctg gtg cgc   271
Glu Ala Phe Asp Asp Ser Gly Lys Val Arg Asp Pro Gln Leu Val Arg
            25                  30                  35

atg ttc ctc atg atg cac ccc tgg tac atc ccc tcc tct cag ctg gcg   319
Met Phe Leu Met Met His Pro Trp Tyr Ile Pro Ser Ser Gln Leu Ala
        40                  45                  50

gcc aag ctg ctc cac atc tac caa caa tcc cgg aag gac aac tcc aat   367
Ala Lys Leu Leu His Ile Tyr Gln Gln Ser Arg Lys Asp Asn Ser Asn
    55                  60                  65

tcc ctg cag gtg aaa acg tgc cac ctg gtc agg tac tgg atc tcc gcc   415
Ser Leu Gln Val Lys Thr Cys His Leu Val Arg Tyr Trp Ile Ser Ala
70                  75                  80                  85

ttc cca gcg gag ttt gac ttg aac ccg gag ttg gct gag cag atc aag   463
Phe Pro Ala Glu Phe Asp Leu Asn Pro Glu Leu Ala Glu Gln Ile Lys
                90                  95                  100

gag ctg aag gct ctg cta gac caa gaa ggg aac cga cgg cac agc agc   511
Glu Leu Lys Ala Leu Leu Asp Gln Glu Gly Asn Arg Arg His Ser Ser
            105                 110                 115

cta atc gac ata gac agc gtc cct acc tac aag tgg aag cgg cag gtg   559
Leu Ile Asp Ile Asp Ser Val Pro Thr Tyr Lys Trp Lys Arg Gln Val
        120                 125                 130
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act cag cgg aac cct gtg gga cag aaa aag cgc aag atg tcc ctg ttg   607
Thr Gln Arg Asn Pro Val Gly Gln Lys Lys Arg Lys Met Ser Leu Leu
    135                 140                 145

ttt gac cac ctg gag ccc atg gag ctg gcg gag cat ctc acc tac ttg   655
Phe Asp His Leu Glu Pro Met Glu Leu Ala Glu His Leu Thr Tyr Leu
150                 155                 160                 165

gag tat cgc tcc ttc tgc aag atc ctg ttt cag gac tat cac agt ttc   703
Glu Tyr Arg Ser Phe Cys Lys Ile Leu Phe Gln Asp Tyr His Ser Phe
                170                 175                 180

gtg act cat ggc tgc act gtg gac aac ccc gtc ctg gag cgg ttc atc   751
Val Thr His Gly Cys Thr Val Asp Asn Pro Val Leu Glu Arg Phe Ile
            185                 190                 195

tcc ctc ttc aac agc gtc tca cag tgg gtg cag ctc atg atc ctc agc   799
Ser Leu Phe Asn Ser Val Ser Gln Trp Val Gln Leu Met Ile Leu Ser
        200                 205                 210

aaa ccc aca gcc ccg cag cgg gcc ctg gtc atc aca cac ttt gtc cac   847
Lys Pro Thr Ala Pro Gln Arg Ala Leu Val Ile Thr His Phe Val His
    215                 220                 225

gtg gcg gag aag ctg cta cag ctg cag aac ttc aac acg ctg atg gca   895
Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe Asn Thr Leu Met Ala
230                 235                 240                 245

gtg gtc ggg ggc ctg agc cac agc tcc atc tcc cgc ctc aag gag acc   943
Val Val Gly Gly Leu Ser His Ser Ser Ile Ser Arg Leu Lys Glu Thr
                250                 255                 260

cac agc cac gtt agc cct gag acc atc aag ctc tgg gag ggt ctc acg   991
His Ser His Val Ser Pro Glu Thr Ile Lys Leu Trp Glu Gly Leu Thr
            265                 270                 275

gaa cta gtg acg gcg aca ggc aac tat ggc aac tac cgg cgt cgg ctg   1039
Glu Leu Val Thr Ala Thr Gly Asn Tyr Gly Asn Tyr Arg Arg Arg Leu
        280                 285                 290

gca gcc tgt gtg ggc ttc cgc ttc ccg atc ctg ggt gtg cac ctc aag   1087
Ala Ala Cys Val Gly Phe Arg Phe Pro Ile Leu Gly Val His Leu Lys
    295                 300                 305

gac ctg gtg gcc ctg cag ctg gca ctg cct gac tgg ctg gac cca gcc   1135
Asp Leu Val Ala Leu Gln Leu Ala Leu Pro Asp Trp Leu Asp Pro Ala
310                 315                 320                 325

cgg acc cgg ctc aac ggg gcc aag atg aag cag ctc ttt agc atc ctg   1183
Arg Thr Arg Leu Asn Gly Ala Lys Met Lys Gln Leu Phe Ser Ile Leu
                330                 335                 340

gag gag ctg gcc atg gtg acc agc ctg cgg cca cca gta cag gcc aac   1231
Glu Glu Leu Ala Met Val Thr Ser Leu Arg Pro Pro Val Gln Ala Asn
            345                 350                 355

ccc gac ctg ctg agc ctg ctc acg gtg tct ctg gat cag tat cag acg   1279
Pro Asp Leu Leu Ser Leu Leu Thr Val Ser Leu Asp Gln Tyr Gln Thr
        360                 365                 370

gag gat gag ctg tac cag ctg tcc ctg cag cgg gag ccg cgc tcc aag   1327
Glu Asp Glu Leu Tyr Gln Leu Ser Leu Gln Arg Glu Pro Arg Ser Lys
    375                 380                 385

tct tcg cca acc agc ccc acg agt tgc acc cca cca ccc cgg ccc ccg   1375
Ser Ser Pro Thr Ser Pro Thr Ser Cys Thr Pro Pro Pro Arg Pro Pro
390                 395                 400                 405

gta ttg gag gag tgg acc tcg gct gcc aaa ccc aag ctg gat cag gcc   1423
Val Leu Glu Glu Trp Thr Ser Ala Ala Lys Pro Lys Leu Asp Gln Ala
                410                 415                 420

ctc gtg gtg gag cac atc gag aag atg gtg gag tct gtg ttc cgg aac   1471
Leu Val Val Glu His Ile Glu Lys Met Val Glu Ser Val Phe Arg Asn
            425                 430                 435
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ttt gac gtc gat ggg gat ggc cac atc tca cag gaa gaa ttc cag atc   1519
Phe Asp Val Asp Gly Asp Gly His Ile Ser Gln Glu Glu Phe Gln Ile
        440                 445                 450

atc cgt ggg aac ttc cct tac ctc agc gcc ttt ggg gac ctc gac cag   1567
Ile Arg Gly Asn Phe Pro Tyr Leu Ser Ala Phe Gly Asp Leu Asp Gln
    455                 460                 465

aac cag gat ggc tgc atc agc agg gag gag atg gtt tcc tat ttc ctg   1615
Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met Val Ser Tyr Phe Leu
470                 475                 480                 485

cgc tcc agc tct gtg ttg ggg ggg cgc atg ggc ttc gta cac aac ttc   1663
Arg Ser Ser Ser Val Leu Gly Gly Arg Met Gly Phe Val His Asn Phe
                490                 495                 500

cag gag agc aac tcc ttg cgc ccc gtc gcc tgc cgc cac tgc aaa gcc   1711
Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys Arg His Cys Lys Ala
            505                 510                 515

ctg atc ctg ggc atc tac aag cag ggc ctc aaa tgc cga gcc tgt gga   1759
Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys Cys Arg Ala Cys Gly
        520                 525                 530

gtg aac tgc cac aag cag tgc aag gat cgc ctg tca gtt gag tgt cgg   1807
Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu Ser Val Glu Cys Arg
    535                 540                 545

cgc agg gcc cag agt gtg agc ctg gag ggg tct gca ccc tca ccc tca   1855
Arg Arg Ala Gln Ser Val Ser Leu Glu Gly Ser Ala Pro Ser Pro Ser
550                 555                 560                 565

ccc atg cac agc cac cat cac cgc gcc ttc agc ttc tct ctg ccc cgc   1903
Pro Met His Ser His His His Arg Ala Phe Ser Phe Ser Leu Pro Arg
                570                 575                 580

cct ggc agg cga ggc tcc agg cct cca gag atc cgt gag gag gag gta   1951
Pro Gly Arg Arg Gly Ser Arg Pro Pro Glu Ile Arg Glu Glu Glu Val
            585                 590                 595

cag acg gtg gag gat ggg gtg ttt gac atc cac ttg taatagatgc        1997
Gln Thr Val Glu Asp Gly Val Phe Asp Ile His Leu
        600                 605

tgtggttgga tcaaggactc attcctgcct tggagaaaat acttcaacca gagcagggag 2057

cctgggggtg tcggggcagg aggctgggga tgggggtggg atatgagggt ggcatgcagc 2117

tgagggcagg gccagggctg gtgtccctaa ggttgtacag actcttgtga atatttgtat 2177

tttccagatg gaataaaaag gcccgtgtaa ttaaccttca aaaaaaaaaa aaaaaaaaa  2236
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<210> 4
<211> 609
<212> PRT
<213> Homo sapiens

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<400> 4
Met Ala Gly Thr Leu Asp Leu Asp Lys Gly Cys Thr Val Glu Glu Leu
  1               5                  10                  15

Leu Arg Gly Cys Ile Glu Ala Phe Asp Asp Ser Gly Lys Val Arg Asp
            20                  25                  30

Pro Gln Leu Val Arg Met Phe Leu Met Met His Pro Trp Tyr Ile Pro
        35                  40                  45

Ser Ser Gln Leu Ala Ala Lys Leu Leu His Ile Tyr Gln Gln Ser Arg
    50                  55                  60

Lys Asp Asn Ser Asn Ser Leu Gln Val Lys Thr Cys His Leu Val Arg
65                  70                  75                  80
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```
Tyr Trp Ile Ser Ala Phe Pro Ala Glu Phe Asp Leu Asn Pro Glu Leu
                 85                  90                  95

Ala Glu Gln Ile Lys Glu Leu Lys Ala Leu Leu Asp Gln Glu Gly Asn
            100                 105                 110

Arg Arg His Ser Ser Leu Ile Asp Ile Asp Ser Val Pro Thr Tyr Lys
        115                 120                 125

Trp Lys Arg Gln Val Thr Gln Arg Asn Pro Val Gly Gln Lys Lys Arg
    130                 135                 140

Lys Met Ser Leu Leu Phe Asp His Leu Glu Pro Met Glu Leu Ala Glu
145                 150                 155                 160

His Leu Thr Tyr Leu Glu Tyr Arg Ser Phe Cys Lys Ile Leu Phe Gln
                165                 170                 175

Asp Tyr His Ser Phe Val Thr His Gly Cys Thr Val Asp Asn Pro Val
            180                 185                 190

Leu Glu Arg Phe Ile Ser Leu Phe Asn Ser Val Ser Gln Trp Val Gln
        195                 200                 205

Leu Met Ile Leu Ser Lys Pro Thr Ala Pro Gln Arg Ala Leu Val Ile
    210                 215                 220

Thr His Phe Val His Val Ala Glu Lys Leu Leu Gln Leu Gln Asn Phe
225                 230                 235                 240

Asn Thr Leu Met Ala Val Val Gly Gly Leu Ser His Ser Ser Ile Ser
                245                 250                 255

Arg Leu Lys Glu Thr His Ser His Val Ser Pro Glu Thr Ile Lys Leu
            260                 265                 270

Trp Glu Gly Leu Thr Glu Leu Val Thr Ala Thr Gly Asn Tyr Gly Asn
        275                 280                 285

Tyr Arg Arg Arg Leu Ala Ala Cys Val Gly Phe Arg Phe Pro Ile Leu
    290                 295                 300

Gly Val His Leu Lys Asp Leu Val Ala Leu Gln Leu Ala Leu Pro Asp
305                 310                 315                 320

Trp Leu Asp Pro Ala Arg Thr Arg Leu Asn Gly Ala Lys Met Lys Gln
                325                 330                 335

Leu Phe Ser Ile Leu Glu Glu Leu Ala Met Val Thr Ser Leu Arg Pro
            340                 345                 350

Pro Val Gln Ala Asn Pro Asp Leu Leu Ser Leu Leu Thr Val Ser Leu
        355                 360                 365

Asp Gln Tyr Gln Thr Glu Asp Glu Leu Tyr Gln Leu Ser Leu Gln Arg
    370                 375                 380

Glu Pro Arg Ser Lys Ser Ser Pro Thr Ser Pro Thr Ser Cys Thr Pro
385                 390                 395                 400

Pro Pro Arg Pro Pro Val Leu Glu Glu Trp Thr Ser Ala Ala Lys Pro
                405                 410                 415

Lys Leu Asp Gln Ala Leu Val Val Glu His Ile Glu Lys Met Val Glu
            420                 425                 430

Ser Val Phe Arg Asn Phe Asp Val Asp Gly Asp Gly His Ile Ser Gln
        435                 440                 445

Glu Glu Phe Gln Ile Ile Arg Gly Asn Phe Pro Tyr Leu Ser Ala Phe
    450                 455                 460

Gly Asp Leu Asp Gln Asn Gln Asp Gly Cys Ile Ser Arg Glu Glu Met
465                 470                 475                 480
```

```
Val Ser Tyr Phe Leu Arg Ser Ser Ser Val Leu Gly Gly Arg Met Gly
                485                 490                 495

Phe Val His Asn Phe Gln Glu Ser Asn Ser Leu Arg Pro Val Ala Cys
            500                 505                 510

Arg His Cys Lys Ala Leu Ile Leu Gly Ile Tyr Lys Gln Gly Leu Lys
        515                 520                 525

Cys Arg Ala Cys Gly Val Asn Cys His Lys Gln Cys Lys Asp Arg Leu
    530                 535                 540

Ser Val Glu Cys Arg Arg Arg Ala Gln Ser Val Ser Leu Glu Gly Ser
545                 550                 555                 560

Ala Pro Ser Pro Ser Pro Met His Ser His His His Arg Ala Phe Ser
                565                 570                 575

Phe Ser Leu Pro Arg Pro Gly Arg Arg Gly Ser Arg Pro Pro Glu Ile
            580                 585                 590

Arg Glu Glu Glu Val Gln Thr Val Glu Asp Gly Val Phe Asp Ile His
        595                 600                 605

Leu
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<210> 5
<211> 3624
<212> DNA
<213> Rattus norvegicus

<220>
<221> CDS
<222> (145)..(2529)
<223> CalDAG-GEFII

<400> 5

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gcgcctgggt cggctcgccg ggctccgaga gtggccggct ccgggctgcg agggccggcg 60

gcggccgagg gatgcgccgc tcccggccga gccgatccca ccgctccagg tgagacggct 120

ccaggggcgc agagagccgc ggcc atg gga acc ctg ggc aag gcg aga gag    171
                          Met Gly Thr Leu Gly Lys Ala Arg Glu
                            1               5

gct ccg cgg aaa cct tgc cat ggc tcc aga gct ggc ccc aaa gga aga   219
Ala Pro Arg Lys Pro Cys His Gly Ser Arg Ala Gly Pro Lys Gly Arg
 10                  15                  20                  25

cta gag gcc aaa tca acc aac agt cct ctc cct gcc cag ccc agc ttg   267
Leu Glu Ala Lys Ser Thr Asn Ser Pro Leu Pro Ala Gln Pro Ser Leu
                 30                  35                  40

gcc cag atc acc cag ttc cga atg atg gtg tcc ctg gga cat ctg gcc   315
Ala Gln Ile Thr Gln Phe Arg Met Met Val Ser Leu Gly His Leu Ala
             45                  50                  55

aaa gga gcc agc ctg gat gat ctt att gac agc tgc att caa tct ttc   363
Lys Gly Ala Ser Leu Asp Asp Leu Ile Asp Ser Cys Ile Gln Ser Phe
         60                  65                  70

gat gca gat gga aac ctg tgt cga agt aac cag ctg tta caa gtc atg   411
Asp Ala Asp Gly Asn Leu Cys Arg Ser Asn Gln Leu Leu Gln Val Met
     75                  80                  85

cta acc atg cac cga atc atc atc tcc tcg gcc gag ctg ctg caa aaa   459
Leu Thr Met His Arg Ile Ile Ile Ser Ser Ala Glu Leu Leu Gln Lys
 90                  95                  100                 105

ctc atg aat cta tat aag gac gcc ctg gaa aag aat tct cca gga att   507
Leu Met Asn Leu Tyr Lys Asp Ala Leu Glu Lys Asn Ser Pro Gly Ile
                110                 115                 120
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```
tgc ttg aag atc tgc tat ttt gtc agg tat tgg ata aca gaa ttc tgg    555
Cys Leu Lys Ile Cys Tyr Phe Val Arg Tyr Trp Ile Thr Glu Phe Trp
            125                 130                 135

atc atg ttc aaa atg gat gcc agc ttg acc agc acc atg gaa gag ttt    603
Ile Met Phe Lys Met Asp Ala Ser Leu Thr Ser Thr Met Glu Glu Phe
        140                 145                 150

cag gac ctg gtg aaa gcc aat ggt gag gag tcc cac tgc cac ctc atc    651
Gln Asp Leu Val Lys Ala Asn Gly Glu Glu Ser His Cys His Leu Ile
    155                 160                 165

gac acg aca caa att aat tct cga gac tgg tcc agg aaa ctg act cag    699
Asp Thr Thr Gln Ile Asn Ser Arg Asp Trp Ser Arg Lys Leu Thr Gln
170                 175                 180                 185

agg ata aaa tca aat acc agc aag aag cgg aaa gtg tcc ctg ctg ttt    747
Arg Ile Lys Ser Asn Thr Ser Lys Lys Arg Lys Val Ser Leu Leu Phe
                190                 195                 200

gac cat ctt gaa cct gaa gaa ctg tct gaa cac ctc acc tac ctt gag    795
Asp His Leu Glu Pro Glu Glu Leu Ser Glu His Leu Thr Tyr Leu Glu
            205                 210                 215

ttc aag tcc ttc cga cgg ata tct ttc tct gat tat cag aat tac ctt    843
Phe Lys Ser Phe Arg Arg Ile Ser Phe Ser Asp Tyr Gln Asn Tyr Leu
        220                 225                 230

gta aac agc tgc gta aag gag aac ccc acc atg gag cgg tcc att gcc    891
Val Asn Ser Cys Val Lys Glu Asn Pro Thr Met Glu Arg Ser Ile Ala
    235                 240                 245

ctg tgc aat ggc atc tcc cag tgg gta caa ctg atg gtt ctc agc cgt    939
Leu Cys Asn Gly Ile Ser Gln Trp Val Gln Leu Met Val Leu Ser Arg
250                 255                 260                 265

ccc acc cca cag ctc cgg gca gag gtc ttc atc aag ttc atc cat gtg    987
Pro Thr Pro Gln Leu Arg Ala Glu Val Phe Ile Lys Phe Ile His Val
                270                 275                 280

gct cag aag ctc cac cag cta cag aac ttc aac acg cta atg gct gtg   1035
Ala Gln Lys Leu His Gln Leu Gln Asn Phe Asn Thr Leu Met Ala Val
            285                 290                 295

atc ggg gga ctg tgt cac agc tcc atc tcc agg ctc aag gag aca agt   1083
Ile Gly Gly Leu Cys His Ser Ser Ile Ser Arg Leu Lys Glu Thr Ser
        300                 305                 310

tca cat gtc cca cat gag atc aat aag gtt ctg ggt gag atg act gaa   1131
Ser His Val Pro His Glu Ile Asn Lys Val Leu Gly Glu Met Thr Glu
    315                 320                 325

ctg ctg tcc tcc tgc aga aac tat gac aac tac agg cga gcc tat ggt   1179
Leu Leu Ser Ser Cys Arg Asn Tyr Asp Asn Tyr Arg Arg Ala Tyr Gly
330                 335                 340                 345

gag tgc acc cac ttc aaa atc ccc ata ctg ggt gtg cac ctc aag gac   1227
Glu Cys Thr His Phe Lys Ile Pro Ile Leu Gly Val His Leu Lys Asp
                350                 355                 360

ctc ata tcc cta tat gaa gcc atg cct gac tac ctg gaa gac ggg aag   1275
Leu Ile Ser Leu Tyr Glu Ala Met Pro Asp Tyr Leu Glu Asp Gly Lys
            365                 370                 375

gtg aat gtc caa aag ctc ctg gcc ctt tac aat cac atc aat gag ttg   1323
Val Asn Val Gln Lys Leu Leu Ala Leu Tyr Asn His Ile Asn Glu Leu
        380                 385                 390

gtc cag ctg cag gac gtg gcc cca cca ttg gat gcc aac aag gac ctg   1371
Val Gln Leu Gln Asp Val Ala Pro Pro Leu Asp Ala Asn Lys Asp Leu
    395                 400                 405

gtg cac ctg ctg acg tta tcc ctg gat cta tac tac acc gaa gat gaa   1419
Val His Leu Leu Thr Leu Ser Leu Asp Leu Tyr Tyr Thr Glu Asp Glu
410                 415                 420                 425
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atc tat gag ctt tcc tac gcc cgt gaa cca agg aac cac agg gcc ccg   1467
Ile Tyr Glu Leu Ser Tyr Ala Arg Glu Pro Arg Asn His Arg Ala Pro
                430                 435                 440

cca ctg aca cct tcg aag cca cca gtt gta gtg gac tgg gcc tct gga   1515
Pro Leu Thr Pro Ser Lys Pro Pro Val Val Val Asp Trp Ala Ser Gly
            445                 450                 455

gtg tct ccc aaa cct gac ccg aag acc atc agc aaa cac gtc caa agg   1563
Val Ser Pro Lys Pro Asp Pro Lys Thr Ile Ser Lys His Val Gln Arg
        460                 465                 470

atg gtg gat tct gtc ttt aag aac tat gat ctc gac cag gat gga tat   1611
Met Val Asp Ser Val Phe Lys Asn Tyr Asp Leu Asp Gln Asp Gly Tyr
    475                 480                 485

atc tct cag gag gag ttt gaa aag att gct gcg agc ttt cca ttt tcc   1659
Ile Ser Gln Glu Glu Phe Glu Lys Ile Ala Ala Ser Phe Pro Phe Ser
490                 495                 500                 505

ttc tgt gtg atg gac aaa gat agg gag ggc ctc atc agc aga gac gag   1707
Phe Cys Val Met Asp Lys Asp Arg Glu Gly Leu Ile Ser Arg Asp Glu
                510                 515                 520

atc aca gcc tac ttc atg agg gcc agc tcc atc tat tcc aag ctg ggc   1755
Ile Thr Ala Tyr Phe Met Arg Ala Ser Ser Ile Tyr Ser Lys Leu Gly
            525                 530                 535

cta ggc ttt cca cac aac ttt caa gag acc act tac ctg aag ccc acc   1803
Leu Gly Phe Pro His Asn Phe Gln Glu Thr Thr Tyr Leu Lys Pro Thr
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ttc tgt gac aac tgt gct ggc ttt ctc tgg ggt gtg atc aag caa ggc   1851
Phe Cys Asp Asn Cys Ala Gly Phe Leu Trp Gly Val Ile Lys Gln Gly
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tat cgc tgt aaa gac tgt ggg atg aac tgc cac aaa cag tgc aaa gac   1899
Tyr Arg Cys Lys Asp Cys Gly Met Asn Cys His Lys Gln Cys Lys Asp
570                 575                 580                 585

ctg gta gtg ttt gag tgc aag aaa cga tcc aag agc ccg gcg gta tcc   1947
Leu Val Val Phe Glu Cys Lys Lys Arg Ser Lys Ser Pro Ala Val Ser
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aca gaa aac atc agc tct gtg gtg cca atg tcc act ctt tgt cca ctg   1995
Thr Glu Asn Ile Ser Ser Val Val Pro Met Ser Thr Leu Cys Pro Leu
            605                 610                 615

gga acc aaa gat ctg ctc cat gca ccc gaa gaa gga tct ttc att ttc   2043
Gly Thr Lys Asp Leu Leu His Ala Pro Glu Glu Gly Ser Phe Ile Phe
        620                 625                 630

cag aat gga gag gtt gtg gac cac agt gag gag agc aag gat agg acc   2091
Gln Asn Gly Glu Val Val Asp His Ser Glu Glu Ser Lys Asp Arg Thr
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atc atg ctc ttg ggc gta tcc tca cag aaa att tca gtt cgg ctg aag   2139
Ile Met Leu Leu Gly Val Ser Ser Gln Lys Ile Ser Val Arg Leu Lys
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agg act gtt gcc cac aag acc acc cag aca gaa tca ttt cct tgg gtt   2187
Arg Thr Val Ala His Lys Thr Thr Gln Thr Glu Ser Phe Pro Trp Val
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ggc ggc gag atg ccc cct ggt cac ttt gtg ctg act tct cca aga aag   2235
Gly Gly Glu Met Pro Pro Gly His Phe Val Leu Thr Ser Pro Arg Lys
            685                 690                 695

tca gca caa ggg gct ctt tat gtg cac agt cca gca tct ccg tgc ccc   2283
Ser Ala Gln Gly Ala Leu Tyr Val His Ser Pro Ala Ser Pro Cys Pro
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Ser Pro Ala Leu Val Arg Lys Arg Ala Phe Val Lys Trp Glu Asn Lys
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gag tcc ctt atc aaa cca aaa cca gag ctt cac ctc agg ctc cgg acc   2379
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730                 735                 740                 745

tac caa gaa ctg gaa cag gag gta aat acc ctg agg gca gat aac gat   2427
Tyr Gln Glu Leu Glu Gln Glu Val Asn Thr Leu Arg Ala Asp Asn Asp
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gct ctg aag atc cag ctg aag tat gca cag aaa caa ata gaa tcc ctg   2475
Ala Leu Lys Ile Gln Leu Lys Tyr Ala Gln Lys Gln Ile Glu Ser Leu
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cag ctt ggc aaa agc aat cac gtc tta gca cag atg gac cac ggt gat   2523
Gln Leu Gly Lys Ser Asn His Val Leu Ala Gln Met Asp His Gly Asp
        780                 785                 790

ggt act taatccagaa attcaaggaa cagaatctgc agacgggttt actgggatct   2579
Gly Thr
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Ser Pro Leu Pro Ala Gln Pro Ser Leu Ala Gln Ile Thr Gln Phe Arg
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Met Met Val Ser Leu Gly His Leu Ala Lys Gly Ala Ser Leu Asp Asp
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Ser Leu Thr Ser Thr Met Glu Glu Phe Gln Asp Leu Val Lys Ala Asn
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Gly Glu Glu Ser His Cys His Leu Ile Asp Thr Thr Gln Ile Asn Ser
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Arg Asp Trp Ser Arg Lys Leu Thr Gln Arg Ile Lys Ser Asn Thr Ser
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Lys Lys Arg Lys Val Ser Leu Leu Phe Asp His Leu Glu Pro Glu Glu
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Leu Ser Glu His Leu Thr Tyr Leu Glu Phe Lys Ser Phe Arg Arg Ile
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Ser Phe Ser Asp Tyr Gln Asn Tyr Leu Val Asn Ser Cys Val Lys Glu
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Asn Pro Thr Met Glu Arg Ser Ile Ala Leu Cys Asn Gly Ile Ser Gln
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Trp Val Gln Leu Met Val Leu Ser Arg Pro Thr Pro Gln Leu Arg Ala
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Glu Val Phe Ile Lys Phe Ile His Val Ala Gln Lys Leu His Gln Leu
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Gln Asn Phe Asn Thr Leu Met Ala Val Ile Gly Gly Leu Cys His Ser
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Ser Ile Ser Arg Leu Lys Glu Thr Ser Ser His Val Pro His Glu Ile
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Tyr Asp Asn Tyr Arg Arg Ala Tyr Gly Glu Cys Thr His Phe Lys Ile
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Pro Ile Leu Gly Val His Leu Lys Asp Leu Ile Ser Leu Tyr Glu Ala
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385 390 395 400

Pro Pro Leu Asp Ala Asn Lys Asp Leu Val His Leu Leu Thr Leu Ser
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Leu Asp Leu Tyr Tyr Thr Glu Asp Glu Ile Tyr Glu Leu Ser Tyr Ala
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Arg Glu Pro Arg Asn His Arg Ala Pro Pro Leu Thr Pro Ser Lys Pro
435 440 445

Pro Val Val Val Asp Trp Ala Ser Gly Val Ser Pro Lys Pro Asp Pro

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Lys Ile Ala Ala Ser Phe Pro Phe Ser Phe Cys Val Met Asp Lys Asp
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Arg Glu Gly Leu Ile Ser Arg Asp Glu Ile Thr Ala Tyr Phe Met Arg
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Ala Ser Ser Ile Tyr Ser Lys Leu Gly Leu Gly Phe Pro His Asn Phe
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Gln Glu Thr Thr Tyr Leu Lys Pro Thr Phe Cys Asp Asn Cys Ala Gly
545                 550                 555                 560

Phe Leu Trp Gly Val Ile Lys Gln Gly Tyr Arg Cys Lys Asp Cys Gly
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Met Asn Cys His Lys Gln Cys Lys Asp Leu Val Val Phe Glu Cys Lys
            580                 585                 590

Lys Arg Ser Lys Ser Pro Ala Val Ser Thr Glu Asn Ile Ser Ser Val
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Val Pro Met Ser Thr Leu Cys Pro Leu Gly Thr Lys Asp Leu Leu His
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Ala Pro Glu Glu Gly Ser Phe Ile Phe Gln Asn Gly Glu Val Val Asp
625                 630                 635                 640

His Ser Glu Glu Ser Lys Asp Arg Thr Ile Met Leu Leu Gly Val Ser
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Ser Gln Lys Ile Ser Val Arg Leu Lys Arg Thr Val Ala His Lys Thr
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His Phe Val Leu Thr Ser Pro Arg Lys Ser Ala Gln Gly Ala Leu Tyr
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Val His Ser Pro Ala Ser Pro Cys Pro Ser Pro Ala Leu Val Arg Lys
705                 710                 715                 720

Arg Ala Phe Val Lys Trp Glu Asn Lys Glu Ser Leu Ile Lys Pro Lys
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Pro Glu Leu His Leu Arg Leu Arg Thr Tyr Gln Glu Leu Glu Gln Glu
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Val Asn Thr Leu Arg Ala Asp Asn Asp Ala Leu Lys Ile Gln Leu Lys
        755                 760                 765

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Met Gly Thr Leu
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Gly Lys Ala Arg Glu Ala Pro Arg Lys Pro Ser His Gly Cys Arg Ala
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Ala Ser Lys Ala Arg Leu Glu Ala Lys Pro Ala Asn Ser Pro Phe Pro
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Ser His Pro Ser Leu Ala His Ile Thr Gln Phe Arg Met Met Val Ser
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ctg gga cat tta gcc aaa gga gcc agc ctg gac gat ctc att gac agc 307
Leu Gly His Leu Ala Lys Gly Ala Ser Leu Asp Asp Leu Ile Asp Ser
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Cys Ile Gln Ser Phe Asp Ala Asp Gly Asn Leu Cys Arg Ser Asn Gln
70 75 80

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Leu Leu Gln Val Met Leu Thr Met His Arg Ile Val Ile Ser Ser Ala
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Glu Leu Leu Gln Lys Val Ile Thr Leu Tyr Lys Asp Ala Leu Ala Lys
105 110 115

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Asn Ser Pro Gly Leu Cys Leu Lys Ile Cys Tyr Phe Val Arg Tyr Trp
120 125 130

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Ile Thr Glu Phe Trp Val Met Phe Lys Met Asp Ala Ser Leu Thr Asp
135 140 145

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Thr Met Glu Glu Phe Gln Glu Leu Val Lys Ala Lys Gly Glu Glu Leu
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165 170 175 180

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Arg Lys Leu Thr Gln Arg Ile Lys Ser Asn Thr Ser Lys Lys Arg Lys
185 190 195

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Val Ser Leu Leu Phe Asp His Leu Glu Pro Glu Glu Leu Ser Glu His
200 205 210

ctc acc tac ctt gag ttc aag tct ttc cgg agg ata tcg ttc tct gat 787
Leu Thr Tyr Leu Glu Phe Lys Ser Phe Arg Arg Ile Ser Phe Ser Asp
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Tyr Gln Asn Tyr Leu Val Asn Ser Cys Val Lys Glu Asn Pro Thr Met
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245 250 255 260

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Met Val Leu Ser Arg Pro Thr Pro Gln Leu Arg Ala Glu Val Phe Ile
265 270 275

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Lys Phe Ile Gln Val Ala Gln Lys Leu His Gln Leu Gln Asn Phe Asn
            280                 285                 290

aca ctg atg gct gtg ata ggt ggg ctg tgt cac agc tca atc tcg agg   1027
Thr Leu Met Ala Val Ile Gly Gly Leu Cys His Ser Ser Ile Ser Arg
        295                 300                 305

ctc aag gag aca agt tcg cat gtc cca cat gaa atc aat aag gtt ctc   1075
Leu Lys Glu Thr Ser Ser His Val Pro His Glu Ile Asn Lys Val Leu
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cgg cga gcc tat gga gag tgc acc gac ttc aag atc ccc att ctg ggt   1171
Arg Arg Ala Tyr Gly Glu Cys Thr Asp Phe Lys Ile Pro Ile Leu Gly
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gtg cat ctc aag gac ctc atc tcc ctg tat gaa gcc atg cct gac tat   1219
Val His Leu Lys Asp Leu Ile Ser Leu Tyr Glu Ala Met Pro Asp Tyr
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ctg ggg gac ggg aaa gtg aac gtc cat aag cta ctg gcc cta tac aat   1267
Leu Gly Asp Gly Lys Val Asn Val His Lys Leu Leu Ala Leu Tyr Asn
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cat atc agt gaa ttg gtc cag ctg caa gag gtg gcc cca ccc ttg gag   1315
His Ile Ser Glu Leu Val Gln Leu Gln Glu Val Ala Pro Pro Leu Glu
    390                 395                 400

gct aac aag gac ttg gta cac ttg ctg acg tta tcc ctg gat ctt tac   1363
Ala Asn Lys Asp Leu Val His Leu Leu Thr Leu Ser Leu Asp Leu Tyr
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tac act gag gat gaa atc tat gag ctt tcc tat gcc cgg gaa cca agg   1411
Tyr Thr Glu Asp Glu Ile Tyr Glu Leu Ser Tyr Ala Arg Glu Pro Arg
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aac cac aga gct cca cca cta aca cct tca aag cca cca gta gta gtg   1459
Asn His Arg Ala Pro Pro Leu Thr Pro Ser Lys Pro Pro Val Val Val
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gac tgg gct tct gga gtg tct ccc aaa cct gat cca aaa acc att agc   1507
Asp Trp Ala Ser Gly Val Ser Pro Lys Pro Asp Pro Lys Thr Ile Ser
        455                 460                 465

aaa cac gtc cag agg atg gtg gat tct gtc ttc aag aac tat gat cac   1555
Lys His Val Gln Arg Met Val Asp Ser Val Phe Lys Asn Tyr Asp His
    470                 475                 480

gac cag gat gga tac att tct cag gaa gaa ttt gaa aag att gct gcg   1603
Asp Gln Asp Gly Tyr Ile Ser Gln Glu Glu Phe Glu Lys Ile Ala Ala
485                 490                 495                 500

agt ttt cca ttt tcc ttc tgt gtg atg gac aaa gac agg gaa ggc ctc   1651
Ser Phe Pro Phe Ser Phe Cys Val Met Asp Lys Asp Arg Glu Gly Leu
                505                 510                 515

atc agc agg gat gag atc aca gcc tac ttc atg aga gcc agc tca atc   1699
Ile Ser Arg Asp Glu Ile Thr Ala Tyr Phe Met Arg Ala Ser Ser Ile
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tat tcc aag ctg ggc ctg ggc ttt cct cac aac ttc caa gag acc acc   1747
Tyr Ser Lys Leu Gly Leu Gly Phe Pro His Asn Phe Gln Glu Thr Thr
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tac ctg aag ccc act ttt tgt gac aac tgt gct gga ttt ctc tgg gga   1795
Tyr Leu Lys Pro Thr Phe Cys Asp Asn Cys Ala Gly Phe Leu Trp Gly
    550                 555                 560

gtg atc aaa caa gga tat cga tgt aaa gac tgc ggg atg aac tgt cac   1843
Val Ile Lys Gln Gly Tyr Arg Cys Lys Asp Cys Gly Met Asn Cys His
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aac cca gta gct ccc aca gag aac aac act tct gtg ggg cca gtg tcc   1939
Asn Pro Val Ala Pro Thr Glu Asn Asn Thr Ser Val Gly Pro Val Ser
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aac ctt tgc tca ttg gga gcc aaa gat ctg ctc cat gca cct gag gaa   1987
Asn Leu Cys Ser Leu Gly Ala Lys Asp Leu Leu His Ala Pro Glu Glu
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gga cct ttt aca ttc cct aat ggg gag gct gtg gaa cat ggt gag gag   2035
Gly Pro Phe Thr Phe Pro Asn Gly Glu Ala Val Glu His Gly Glu Glu
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Ser Lys Asp Arg Thr Ile Met Leu Met Gly Val Ser Ser Gln Lys Ile
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Ser Ser Pro Arg Lys Thr Ala Gln Asp Thr Leu Tyr Val Leu Pro Ser
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Pro Thr Ser Pro Cys Pro Ser Pro Val Leu Val Arg Lys Arg Ala Phe
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gtc aag tgg gag aat aaa gac tcc ctc ata aaa tca aag gag gag ctc   2323
Val Lys Trp Glu Asn Lys Asp Ser Leu Ile Lys Ser Lys Glu Glu Leu
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act ctg aaa gca gat aat gat gcc cta aag atc caa ctg aaa tat gca   2419
Thr Leu Lys Ala Asp Asn Asp Ala Leu Lys Ile Gln Leu Lys Tyr Ala
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Gln Lys Lys Ile Glu Ser Leu Gln Leu Glu Lys Ser Asn His Val Leu
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Ser Pro Phe Pro Ser His Pro Ser Leu Ala His Ile Thr Gln Phe Arg
35 40 45

Met Met Val Ser Leu Gly His Leu Ala Lys Gly Ala Ser Leu Asp Asp
50 55 60

Leu Ile Asp Ser Cys Ile Gln Ser Phe Asp Ala Asp Gly Asn Leu Cys
65 70 75 80

Arg Ser Asn Gln Leu Leu Gln Val Met Leu Thr Met His Arg Ile Val
85 90 95

Ile Ser Ser Ala Glu Leu Leu Gln Lys Val Ile Thr Leu Tyr Lys Asp
100 105 110

Ala Leu Ala Lys Asn Ser Pro Gly Leu Cys Leu Lys Ile Cys Tyr Phe
115 120 125

Val Arg Tyr Trp Ile Thr Glu Phe Trp Val Met Phe Lys Met Asp Ala
130 135 140

Ser Leu Thr Asp Thr Met Glu Glu Phe Gln Glu Leu Val Lys Ala Lys
145 150 155 160

Gly Glu Glu Leu His Cys Arg Leu Ile Asp Thr Thr Gln Ile Asn Ala
165 170 175

Arg Asp Trp Ser Arg Lys Leu Thr Gln Arg Ile Lys Ser Asn Thr Ser
180 185 190

Lys Lys Arg Lys Val Ser Leu Leu Phe Asp His Leu Glu Pro Glu Glu
195 200 205

Leu Ser Glu His Leu Thr Tyr Leu Glu Phe Lys Ser Phe Arg Arg Ile
210 215 220

Ser Phe Ser Asp Tyr Gln Asn Tyr Leu Val Asn Ser Cys Val Lys Glu
225 230 235 240

Asn Pro Thr Met Glu Arg Ser Ile Ala Leu Cys Asn Gly Ile Ser Gln
245 250 255

Trp Val Gln Leu Met Val Leu Ser Arg Pro Thr Pro Gln Leu Arg Ala
260 265 270

Glu Val Phe Ile Lys Phe Ile Gln Val Ala Gln Lys Leu His Gln Leu
275 280 285

Gln Asn Phe Asn Thr Leu Met Ala Val Ile Gly Gly Leu Cys His Ser
290 295 300

Ser Ile Ser Arg Leu Lys Glu Thr Ser Ser His Val Pro His Glu Ile
305 310 315 320

Asn Lys Val Leu Gly Glu Met Thr Glu Leu Leu Ser Ser Ser Arg Asn
325 330 335

Tyr Asp Asn Tyr Arg Arg Ala Tyr Gly Glu Cys Thr Asp Phe Lys Ile
340 345 350

Pro Ile Leu Gly Val His Leu Lys Asp Leu Ile Ser Leu Tyr Glu Ala
355 360 365

Met Pro Asp Tyr Leu Gly Asp Gly Lys Val Asn Val His Lys Leu Leu
370 375 380

Ala Leu Tyr Asn His Ile Ser Glu Leu Val Gln Leu Gln Glu Val Ala
385 390 395 400

Pro Pro Leu Glu Ala Asn Lys Asp Leu Val His Leu Leu Thr Leu Ser
405 410 415

Leu Asp Leu Tyr Tyr Thr Glu Asp Glu Ile Tyr Glu Leu Ser Tyr Ala
420 425 430

Arg Glu Pro Arg Asn His Arg Ala Pro Pro Leu Thr Pro Ser Lys Pro
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Pro Val Val Val Asp Trp Ala Ser Gly Val Ser Pro Lys Pro Asp Pro
450 455 460

Lys Thr Ile Ser Lys His Val Gln Arg Met Val Asp Ser Val Phe Lys
465 470 475 480

Asn Tyr Asp His Asp Gln Asp Gly Tyr Ile Ser Gln Glu Glu Phe Glu
485 490 495

Lys Ile Ala Ala Ser Phe Pro Phe Ser Phe Cys Val Met Asp Lys Asp
500 505 510

Arg Glu Gly Leu Ile Ser Arg Asp Glu Ile Thr Ala Tyr Phe Met Arg
515 520 525

Ala Ser Ser Ile Tyr Ser Lys Leu Gly Leu Gly Phe Pro His Asn Phe
530 535 540

Gln Glu Thr Thr Tyr Leu Lys Pro Thr Phe Cys Asp Asn Cys Ala Gly
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Phe Leu Trp Gly Val Ile Lys Gln Gly Tyr Arg Cys Lys Asp Cys Gly
565 570 575

Met Asn Cys His Lys Gln Cys Lys Asp Leu Val Val Phe Glu Cys Lys
580 585 590

Lys Arg Ala Lys Asn Pro Val Ala Pro Thr Glu Asn Asn Thr Ser Val
595 600 605

Gly Pro Val Ser Asn Leu Cys Ser Leu Gly Ala Lys Asp Leu Leu His
610 615 620

Ala Pro Glu Glu Gly Pro Phe Thr Phe Pro Asn Gly Glu Ala Val Glu
625 630 635 640

His Gly Glu Glu Ser Lys Asp Arg Thr Ile Met Leu Met Gly Val Ser
645 650 655

Ser Gln Lys Ile Ser Leu Arg Leu Lys Arg Ala Val Ala His Lys Ala
660 665 670

Thr Gln Thr Glu Ser Gln Pro Trp Ile Gly Ser Glu Gly Pro Ser Gly
675 680 685

Pro Phe Val Leu Ser Ser Pro Arg Lys Thr Ala Gln Asp Thr Leu Tyr
690 695 700

Val Leu Pro Ser Pro Thr Ser Pro Cys Pro Ser Pro Val Leu Val Arg
705 710 715 720

Lys Arg Ala Phe Val Lys Trp Glu Asn Lys Asp Ser Leu Ile Lys Ser
725 730 735

Lys Glu Glu Leu Arg His Leu Arg Leu Pro Thr Tyr Gln Glu Leu Glu
740 745 750

Gln Glu Ile Asn Thr Leu Lys Ala Asp Asn Asp Ala Leu Lys Ile Gln
755 760 765

Leu Lys Tyr Ala Gln Lys Lys Ile Glu Ser Leu Gln Leu Glu Lys Ser
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ggtggagagt ccagctgtgg gggcaccgca ggtgcgaggt ctcccggacg tggttccgga 180

gggcacgctg ctcaat atg gtg ctg aag aga atg cac cgt ccc cgg tgc tgc 232
Met Val Leu Lys Arg Met His Arg Pro Arg Cys Cys
1 5 10

tct tac cag cta gtg ttc gag cac cgg cgc cca agc tgc atc cag gga 280
Ser Tyr Gln Leu Val Phe Glu His Arg Arg Pro Ser Cys Ile Gln Gly
15 20 25

ctt cgc tgg acg cca ctt acc aac agt gag ggc tcc ctg gac ttc aga 328
Leu Arg Trp Thr Pro Leu Thr Asn Ser Glu Gly Ser Leu Asp Phe Arg
30 35 40

gtg agc ctg gag cag gcc acc aca gag cat gtg cac aag gcc ggg aag 376
Val Ser Leu Glu Gln Ala Thr Thr Glu His Val His Lys Ala Gly Lys
45 50 55 60

ctc ctg tac cgt cat ctc ttg gca acg tac cct acc ctc atc cga gac 424
Leu Leu Tyr Arg His Leu Leu Ala Thr Tyr Pro Thr Leu Ile Arg Asp
65 70 75

aga aaa tac cat ctg cga cta cat cgg cag tgc tgc tct ggc cgg gag 472
Arg Lys Tyr His Leu Arg Leu His Arg Gln Cys Cys Ser Gly Arg Glu
80 85 90

cta gtg gat ggg atc ttg gct ctg ggt ctt ggg gtc cac tca cgg agc 520
Leu Val Asp Gly Ile Leu Ala Leu Gly Leu Gly Val His Ser Arg Ser
95 100 105

caa gct gtg ggc atc tgc cag gtg ttg ctg gat gag ggt gcc ctt tgc 568
Gln Ala Val Gly Ile Cys Gln Val Leu Leu Asp Glu Gly Ala Leu Cys
110 115 120

cat gta aaa cat gac tgg acc ttc cag gac cga gac gcc caa ttc tac 616
His Val Lys His Asp Trp Thr Phe Gln Asp Arg Asp Ala Gln Phe Tyr
125 130 135 140

aga ttc cct gga ccg gag ccc cag cct gca gga act cat gac gtg gaa 664
Arg Phe Pro Gly Pro Glu Pro Gln Pro Ala Gly Thr His Asp Val Glu
145 150 155

gag gag ctt gtt gag gca atg gcc cta ctc tcc cag cga ggg cct gat 712
Glu Glu Leu Val Glu Ala Met Ala Leu Leu Ser Gln Arg Gly Pro Asp
160 165 170

gcc cta ctc act gtt gca ctc cgg aag tcc ccg ggt cag cgt aca gat 760
Ala Leu Leu Thr Val Ala Leu Arg Lys Ser Pro Gly Gln Arg Thr Asp
175 180 185

gaa gag ctg gac ctc atc ttc gag gag ctc gta cat atc aag gcg gtg 808
Glu Glu Leu Asp Leu Ile Phe Glu Glu Leu Val His Ile Lys Ala Val
190 195 200

gct cac ctt tct aac tcg gtg aaa cgg gaa cta gct gct gtt ctg ctc 856
Ala His Leu Ser Asn Ser Val Lys Arg Glu Leu Ala Ala Val Leu Leu
205 210 215 220

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ttt gaa cca cac agc aag gca gga act gtg ttg ttc agc cag ggg gac   904
Phe Glu Pro His Ser Lys Ala Gly Thr Val Leu Phe Ser Gln Gly Asp
                225                 230                 235

aag ggt acc tca tgg tac att atc tgg aag gga tct gtc aat gtg gtg   952
Lys Gly Thr Ser Trp Tyr Ile Ile Trp Lys Gly Ser Val Asn Val Val
            240                 245                 250

acc cgt ggc aag ggg ctg gtg acc acg ttg cac gag gga gat gac ttt  1000
Thr Arg Gly Lys Gly Leu Val Thr Thr Leu His Glu Gly Asp Asp Phe
        255                 260                 265

gga cag ctg gct ctg gtg aac gac gca cct cga gca gcc acc atc atc  1048
Gly Gln Leu Ala Leu Val Asn Asp Ala Pro Arg Ala Ala Thr Ile Ile
    270                 275                 280

ctt cga gaa aat aac tgt cac ttt ctg cgt gtg gac aag cag gac ttc  1096
Leu Arg Glu Asn Asn Cys His Phe Leu Arg Val Asp Lys Gln Asp Phe
285                 290                 295                 300

aac cgc atc atc aag gat gtg gaa gca aaa acc atg aga ctg gaa gaa  1144
Asn Arg Ile Ile Lys Asp Val Glu Ala Lys Thr Met Arg Leu Glu Glu
                305                 310                 315

cac ggc aaa gtg gtg tta gtt ttg gag aga acc tct cag ggt gct ggc  1192
His Gly Lys Val Val Leu Val Leu Glu Arg Thr Ser Gln Gly Ala Gly
            320                 325                 330

cct tcc cgc cct ccg acc cca ggc agg aac cga tat acg gta atg tct  1240
Pro Ser Arg Pro Pro Thr Pro Gly Arg Asn Arg Tyr Thr Val Met Ser
        335                 340                 345

ggc acc cca gag aaa atc cta gaa ctc ctg ttg gag gct atg aga ccg  1288
Gly Thr Pro Glu Lys Ile Leu Glu Leu Leu Leu Glu Ala Met Arg Pro
    350                 355                 360

gat tcc agt gct cat gac cca aca gag aca ttc ctc agt gac ttc ctg  1336
Asp Ser Ser Ala His Asp Pro Thr Glu Thr Phe Leu Ser Asp Phe Leu
365                 370                 375                 380

ctg acg cac agt gtc ttc atg ccc tgc aca cag ctc ttt gcc gcc ctc  1384
Leu Thr His Ser Val Phe Met Pro Cys Thr Gln Leu Phe Ala Ala Leu
                385                 390                 395

ctg cac cac ttc cac gtg gag cca tca gag cct gcc ggg ggc agc gag  1432
Leu His His Phe His Val Glu Pro Ser Glu Pro Ala Gly Gly Ser Glu
            400                 405                 410

cag gaa cgc agc acc tac atc tgc aac aag agg cag cag att ctg cgt  1480
Gln Glu Arg Ser Thr Tyr Ile Cys Asn Lys Arg Gln Gln Ile Leu Arg
        415                 420                 425

ctg gtc agc cgg tgg gtg gcc ctc tac agc ccc atg ctc cgc tca gat  1528
Leu Val Ser Arg Trp Val Ala Leu Tyr Ser Pro Met Leu Arg Ser Asp
    430                 435                 440

ccc gtg gcc acc agc ttc ctc cag aaa ctc tca gac ctg gtg agc aga  1576
Pro Val Ala Thr Ser Phe Leu Gln Lys Leu Ser Asp Leu Val Ser Arg
445                 450                 455                 460

gat acc cga ctt agc aac ttg ctg agg gaa cag tat ccg gag aga cgg  1624
Asp Thr Arg Leu Ser Asn Leu Leu Arg Glu Gln Tyr Pro Glu Arg Arg
                465                 470                 475

cga cac cac agg ttg gag aat ggc tgt ggg aat gta tct cct cag acc  1672
Arg His His Arg Leu Glu Asn Gly Cys Gly Asn Val Ser Pro Gln Thr
            480                 485                 490

aag gcc cgg aat gca cct gtt tgg ttt cct aac cat gag gaa ccc ctc  1720
Lys Ala Arg Asn Ala Pro Val Trp Phe Pro Asn His Glu Glu Pro Leu
        495                 500                 505

cca agc agc gct ggg gcc atc cga gtc ggg gac aaa gtc ccc tat gat  1768
Pro Ser Ser Ala Gly Ala Ile Arg Val Gly Asp Lys Val Pro Tyr Asp
    510                 515                 520
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atc tgc aga ccc gac cac tcg gtg ctg acc ctg cac ctg ccg gtg aca   1816
Ile Cys Arg Pro Asp His Ser Val Leu Thr Leu His Leu Pro Val Thr
525                 530                 535                 540

gcc tcc gtg agg gaa gtg atg gca gct ttg gcc cat gag gac cac tgg   1864
Ala Ser Val Arg Glu Val Met Ala Ala Leu Ala His Glu Asp His Trp
                545                 550                 555

acc aag ggg cag gtg ctg gta aag gtc aat tct gcc ggt gat gtc gtt   1912
Thr Lys Gly Gln Val Leu Val Lys Val Asn Ser Ala Gly Asp Val Val
            560                 565                 570

ggc ttg cag cca gat gcc cgc ggt gtg gcc aca tcc ctg ggg ctc aat   1960
Gly Leu Gln Pro Asp Ala Arg Gly Val Ala Thr Ser Leu Gly Leu Asn
        575                 580                 585

gag cgg atc ttt gtt gtc gac cca cag gaa gtg cac gag ctg acc cca   2008
Glu Arg Ile Phe Val Val Asp Pro Gln Glu Val His Glu Leu Thr Pro
    590                 595                 600

cac cct gag cag ctg ggg ccc act ctg ggt tct tct gag atg ctg gac   2056
His Pro Glu Gln Leu Gly Pro Thr Leu Gly Ser Ser Glu Met Leu Asp
605                 610                 615                 620

cta gtg agt gcc aag gac ctg gca ggc cag ctc aca gag cat gac tgg   2104
Leu Val Ser Ala Lys Asp Leu Ala Gly Gln Leu Thr Glu His Asp Trp
                625                 630                 635

aac ctc ttc aac agg atc cac cag gtg gag ctg atc cac tat gta ctg   2152
Asn Leu Phe Asn Arg Ile His Gln Val Glu Leu Ile His Tyr Val Leu
            640                 645                 650

ggc ccc cag cac ctg cgg gac gtc acc act gca aac ctg gag cgc ttc   2200
Gly Pro Gln His Leu Arg Asp Val Thr Thr Ala Asn Leu Glu Arg Phe
        655                 660                 665

atg cga cgc ttc aac gag ctg cag tac tgg gtg gcc acc gaa ctc tgt   2248
Met Arg Arg Phe Asn Glu Leu Gln Tyr Trp Val Ala Thr Glu Leu Cys
    670                 675                 680

ctc tgc ccg gtt cct ggc ccc cgg gct cag cta ctc cgg aag ttc atc   2296
Leu Cys Pro Val Pro Gly Pro Arg Ala Gln Leu Leu Arg Lys Phe Ile
685                 690                 695                 700

aag ctg gca gcc cac ctc aag gag cag aag aat ctc aac tct ttc ttt   2344
Lys Leu Ala Ala His Leu Lys Glu Gln Lys Asn Leu Asn Ser Phe Phe
                705                 710                 715

gcg gtc atg ttt ggc ctc agc aac tcg gcc atc agc cgc ctg gcc cac   2392
Ala Val Met Phe Gly Leu Ser Asn Ser Ala Ile Ser Arg Leu Ala His
            720                 725                 730

acc tgg gag cgt ctg ccc cat aaa gta cgg aag ctg tac tcg gcc ctg   2440
Thr Trp Glu Arg Leu Pro His Lys Val Arg Lys Leu Tyr Ser Ala Leu
        735                 740                 745

gaa agg ttg ctg gac cct tcc tgg aac cac cga gtg tac cga ttg gct   2488
Glu Arg Leu Leu Asp Pro Ser Trp Asn His Arg Val Tyr Arg Leu Ala
    750                 755                 760

ctc acc aag ctc tct cct cct gtc atc cct ttc atg ccc ctg cta ctc   2536
Leu Thr Lys Leu Ser Pro Pro Val Ile Pro Phe Met Pro Leu Leu Leu
765                 770                 775                 780

aaa gac atg acc ttc att cat gaa ggg aac cac aca ctg gta gaa aac   2584
Lys Asp Met Thr Phe Ile His Glu Gly Asn His Thr Leu Val Glu Asn
                785                 790                 795

ctc atc aac ttt gag aag atg cga atg atg gcc aga gcc gtg cgg atg   2632
Leu Ile Asn Phe Glu Lys Met Arg Met Met Ala Arg Ala Val Arg Met
            800                 805                 810

ctc cac cac tgc cga agc cac agc acc gcg cct cta tca cca ctc aga   2680
Leu His His Cys Arg Ser His Ser Thr Ala Pro Leu Ser Pro Leu Arg
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        815                 820                 825

agc cgg gtt tcc cac atc cac gag gac agc cag gca tca aga atc tcc   2728
Ser Arg Val Ser His Ile His Glu Asp Ser Gln Ala Ser Arg Ile Ser
    830                 835                 840

aca tgt tcc gag cag tcc ctg agc acc cgg agt cca gcc agc acc tgg   2776
Thr Cys Ser Glu Gln Ser Leu Ser Thr Arg Ser Pro Ala Ser Thr Trp
845                 850                 855                 860

gct tat gtc cag cag ctg aag gtc att gac aac cag cgg gaa ctg tcc   2824
Ala Tyr Val Gln Gln Leu Lys Val Ile Asp Asn Gln Arg Glu Leu Ser
                865                 870                 875

cgc ctc tcc cgg gaa ctg gaa cca tgaggaagga ctggctggag caggcacttc  2878
Arg Leu Ser Arg Glu Leu Glu Pro
            880

tctcagagaa agccagagcc tgtgcaacca agaggtccag aggccagcca cagctgggca 2938

gggctctcca cagagcggac tcaaggccct ggagtgggca gtgtcgaggc agctgtcctc 2998

tgtgatgact gtcagctgtg aagatctttg atgttcacgg ccaaggaaaa ggggccattg 3058

aggccccaga ggggtaggag agctgggagg tgcaggactc tggttcagta gagagccctc 3118

ccaccgggct ttctgcatgt ctgtatgtct gtacatgcag ctgtgtgtcc tggatgccag 3178

gccctgtgct tgtattcaca ggagccagcg agctcacatc tgcatggtgt gtgtgtgtgt 3238

gtgtgtgtgt gtgtgtgtgt gtgtgtgtga gcactgggtg gtgccttgcc tgggagaggg 3298

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aaaaaaaaaa aaaaa                                                  3373
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Pro Leu Thr Asn Ser Glu Gly Ser Leu Asp Phe Arg Val Ser Leu Glu
        35                  40                  45

Gln Ala Thr Thr Glu His Val His Lys Ala Gly Lys Leu Leu Tyr Arg
    50                  55                  60

His Leu Leu Ala Thr Tyr Pro Thr Leu Ile Arg Asp Arg Lys Tyr His
65                  70                  75                  80

Leu Arg Leu His Arg Gln Cys Cys Ser Gly Arg Glu Leu Val Asp Gly
                85                  90                  95

Ile Leu Ala Leu Gly Leu Gly Val His Ser Arg Ser Gln Ala Val Gly
            100                 105                 110

Ile Cys Gln Val Leu Leu Asp Glu Gly Ala Leu Cys His Val Lys His
        115                 120                 125

Asp Trp Thr Phe Gln Asp Arg Asp Ala Gln Phe Tyr Arg Phe Pro Gly
    130                 135                 140

Pro Glu Pro Gln Pro Ala Gly Thr His Asp Val Glu Glu Glu Leu Val
145                 150                 155                 160

Glu Ala Met Ala Leu Leu Ser Gln Arg Gly Pro Asp Ala Leu Leu Thr
                165                 170                 175
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Val Ala Leu Arg Lys Ser Pro Gly Gln Arg Thr Asp Glu Glu Leu Asp
180 185 190

Leu Ile Phe Glu Glu Leu Val His Ile Lys Ala Val Ala His Leu Ser
195 200 205

Asn Ser Val Lys Arg Glu Leu Ala Ala Val Leu Leu Phe Glu Pro His
210 215 220

Ser Lys Ala Gly Thr Val Leu Phe Ser Gln Gly Asp Lys Gly Thr Ser
225 230 235 240

Trp Tyr Ile Ile Trp Lys Gly Ser Val Asn Val Val Thr Arg Gly Lys
245 250 255

Gly Leu Val Thr Thr Leu His Glu Gly Asp Asp Phe Gly Gln Leu Ala
260 265 270

Leu Val Asn Asp Ala Pro Arg Ala Ala Thr Ile Ile Leu Arg Glu Asn
275 280 285

Asn Cys His Phe Leu Arg Val Asp Lys Gln Asp Phe Asn Arg Ile Ile
290 295 300

Lys Asp Val Glu Ala Lys Thr Met Arg Leu Glu Glu His Gly Lys Val
305 310 315 320

Val Leu Val Leu Glu Arg Thr Ser Gln Gly Ala Gly Pro Ser Arg Pro
325 330 335

Pro Thr Pro Gly Arg Asn Arg Tyr Thr Val Met Ser Gly Thr Pro Glu
340 345 350

Lys Ile Leu Glu Leu Leu Leu Glu Ala Met Arg Pro Asp Ser Ser Ala
355 360 365

His Asp Pro Thr Glu Thr Phe Leu Ser Asp Phe Leu Leu Thr His Ser
370 375 380

Val Phe Met Pro Cys Thr Gln Leu Phe Ala Ala Leu Leu His His Phe
385 390 395 400

His Val Glu Pro Ser Glu Pro Ala Gly Gly Ser Glu Gln Glu Arg Ser
405 410 415

Thr Tyr Ile Cys Asn Lys Arg Gln Gln Ile Leu Arg Leu Val Ser Arg
420 425 430

Trp Val Ala Leu Tyr Ser Pro Met Leu Arg Ser Asp Pro Val Ala Thr
435 440 445

Ser Phe Leu Gln Lys Leu Ser Asp Leu Val Ser Arg Asp Thr Arg Leu
450 455 460

Ser Asn Leu Leu Arg Glu Gln Tyr Pro Glu Arg Arg Arg His His Arg
465 470 475 480

Leu Glu Asn Gly Cys Gly Asn Val Ser Pro Gln Thr Lys Ala Arg Asn
485 490 495

Ala Pro Val Trp Phe Pro Asn His Glu Glu Pro Leu Pro Ser Ser Ala
500 505 510

Gly Ala Ile Arg Val Gly Asp Lys Val Pro Tyr Asp Ile Cys Arg Pro
515 520 525

Asp His Ser Val Leu Thr Leu His Leu Pro Val Thr Ala Ser Val Arg
530 535 540

Glu Val Met Ala Ala Leu Ala His Glu Asp His Trp Thr Lys Gly Gln
545 550 555 560

Val Leu Val Lys Val Asn Ser Ala Gly Asp Val Val Gly Leu Gln Pro
565 570 575

Asp Ala Arg Gly Val Ala Thr Ser Leu Gly Leu Asn Glu Arg Ile Phe
580 585 590

Val Val Asp Pro Gln Glu Val His Glu Leu Thr Pro His Pro Glu Gln
595 600 605

Leu Gly Pro Thr Leu Gly Ser Ser Glu Met Leu Asp Leu Val Ser Ala
610 615 620

Lys Asp Leu Ala Gly Gln Leu Thr Glu His Asp Trp Asn Leu Phe Asn
625 630 635 640

Arg Ile His Gln Val Glu Leu Ile His Tyr Val Leu Gly Pro Gln His
645 650 655

Leu Arg Asp Val Thr Thr Ala Asn Leu Glu Arg Phe Met Arg Arg Phe
660 665 670

Asn Glu Leu Gln Tyr Trp Val Ala Thr Glu Leu Cys Leu Cys Pro Val
675 680 685

Pro Gly Pro Arg Ala Gln Leu Leu Arg Lys Phe Ile Lys Leu Ala Ala
690 695 700

His Leu Lys Glu Gln Lys Asn Leu Asn Ser Phe Phe Ala Val Met Phe
705 710 715 720

Gly Leu Ser Asn Ser Ala Ile Ser Arg Leu Ala His Thr Trp Glu Arg
725 730 735

Leu Pro His Lys Val Arg Lys Leu Tyr Ser Ala Leu Glu Arg Leu Leu
740 745 750

Asp Pro Ser Trp Asn His Arg Val Tyr Arg Leu Ala Leu Thr Lys Leu
755 760 765

Ser Pro Pro Val Ile Pro Phe Met Pro Leu Leu Leu Lys Asp Met Thr
770 775 780

Phe Ile His Glu Gly Asn His Thr Leu Val Glu Asn Leu Ile Asn Phe
785 790 795 800

Glu Lys Met Arg Met Met Ala Arg Ala Val Arg Met Leu His His Cys
805 810 815

Arg Ser His Ser Thr Ala Pro Leu Ser Pro Leu Arg Ser Arg Val Ser
820 825 830

His Ile His Glu Asp Ser Gln Ala Ser Arg Ile Ser Thr Cys Ser Glu
835 840 845

Gln Ser Leu Ser Thr Arg Ser Pro Ala Ser Thr Trp Ala Tyr Val Gln
850 855 860

Gln Leu Lys Val Ile Asp Asn Gln Arg Glu Leu Ser Arg Leu Ser Arg
865 870 875 880

Glu Leu Glu Pro

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ggcaggtggg cctggctgtg gaggatagcc cagctctggg agcaccgcgg gtgggagccc 180

tccctgacgt ggtgccggag gggacactac tcaac atg gtg ttg aga agg atg    233
                                      Met Val Leu Arg Arg Met
                                        1               5

cac cgg ccc cga agc tgc tcc tac cag ctg ctg ctg gag cac cag cat    281
His Arg Pro Arg Ser Cys Ser Tyr Gln Leu Leu Leu Glu His Gln His
            10                  15                  20

ccg agc tgc atc cag ggg ctg cgc tgg aca cca ctc acc aac agc gag    329
Pro Ser Cys Ile Gln Gly Leu Arg Trp Thr Pro Leu Thr Asn Ser Glu
        25                  30                  35

gag tcc ctg gat ttc agc gag agc ctg gag cag gcc tcc aca gag cgg    377
Glu Ser Leu Asp Phe Ser Glu Ser Leu Glu Gln Ala Ser Thr Glu Arg
    40                  45                  50

gtg ctc agg gct ggg agg cag ctg cat cag cat cta ctg gcc acc tgc    425
Val Leu Arg Ala Gly Arg Gln Leu His Gln His Leu Leu Ala Thr Cys
55                  60                  65                  70

cca aac ctc atc cga gac cgg aag tac cac ctt agg ctc tat cgg cag    473
Pro Asn Leu Ile Arg Asp Arg Lys Tyr His Leu Arg Leu Tyr Arg Gln
                75                  80                  85

tgc tgc tct ggc cgg gag ctg gtg gat ggg atc ttg gcc ctg gga ctt    521
Cys Cys Ser Gly Arg Glu Leu Val Asp Gly Ile Leu Ala Leu Gly Leu
            90                  95                  100

ggg gtc cat tcc cgg agc caa gtt gtg gga atc tgc cag gtg ctg ctg    569
Gly Val His Ser Arg Ser Gln Val Val Gly Ile Cys Gln Val Leu Leu
        105                 110                 115

gat gaa ggt gcc ctc tgc cat gtg aaa cac gac tgg gcc ttc cag gac    617
Asp Glu Gly Ala Leu Cys His Val Lys His Asp Trp Ala Phe Gln Asp
    120                 125                 130

cga gat gcc caa ttc tac cgg ttc ccc ggg ccc gag ccc gag ccc gtg    665
Arg Asp Ala Gln Phe Tyr Arg Phe Pro Gly Pro Glu Pro Glu Pro Val
135                 140                 145                 150

gga act cat gag atg gag gag gag ttg gcc gaa gct gtg gcc ctg ctc    713
Gly Thr His Glu Met Glu Glu Glu Leu Ala Glu Ala Val Ala Leu Leu
                155                 160                 165

tcc cag cgg ggg cct gac gcc ctg ctc act gtg gca ctt cga aag ccc    761
Ser Gln Arg Gly Pro Asp Ala Leu Leu Thr Val Ala Leu Arg Lys Pro
            170                 175                 180

cca ggt cag cgc acg gat gaa gag ctg gac ctc atc ttt gag gag ctg    809
Pro Gly Gln Arg Thr Asp Glu Glu Leu Asp Leu Ile Phe Glu Glu Leu
        185                 190                 195

ctg cac atc aag gct gtg gcc cac ctc tcc aac tcg gtg aag cga gaa    857
Leu His Ile Lys Ala Val Ala His Leu Ser Asn Ser Val Lys Arg Glu
    200                 205                 210

tta gcg gct gtt ctg ctc ttt gaa cca cac agc aag gca ggg acc gtg    905
Leu Ala Ala Val Leu Leu Phe Glu Pro His Ser Lys Ala Gly Thr Val
215                 220                 225                 230

ttg ttc agc cag ggg gac aag ggc act tcg tgg tac att atc tgg aag    953
Leu Phe Ser Gln Gly Asp Lys Gly Thr Ser Trp Tyr Ile Ile Trp Lys
                235                 240                 245

gga tct gtc aac gtg gtg acc cat ggc aag ggg ctg gtg acc acc ctg   1001
Gly Ser Val Asn Val Val Thr His Gly Lys Gly Leu Val Thr Thr Leu
            250                 255                 260

cat gag gga gat gat ttt gga cag ctg gct ctg gtg aat gat gca ccc   1049
His Glu Gly Asp Asp Phe Gly Gln Leu Ala Leu Val Asn Asp Ala Pro
        265                 270                 275
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cgg gca gcc acc atc atc ctg cga gaa tac aac tgt cat ttc ctg cgt   1097
Arg Ala Ala Thr Ile Ile Leu Arg Glu Tyr Asn Cys His Phe Leu Arg
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gtg gac aag cag gac ttc aac cgt atc atc aag gat gtg gag gca aag   1145
Val Asp Lys Gln Asp Phe Asn Arg Ile Ile Lys Asp Val Glu Ala Lys
295                 300                 305                 310

acc atg cgg ctg gaa gaa cat ggc aaa gtg gtg ctg gtg ctg gag aga   1193
Thr Met Arg Leu Glu Glu His Gly Lys Val Val Leu Val Leu Glu Arg
                315                 320                 325

gcc tct cag ggc gcc ggc cct tcc cga ccc cca acc cca ggc agg aac   1241
Ala Ser Gln Gly Ala Gly Pro Ser Arg Pro Pro Thr Pro Gly Arg Asn
            330                 335                 340

cgg tat aca gtg atg tct ggc act cca gat aag atc cta gag ctt ctg   1289
Arg Tyr Thr Val Met Ser Gly Thr Pro Asp Lys Ile Leu Glu Leu Leu
        345                 350                 355

ttg gag gcc atg gga cta gat tcc agt gct cat gac cca aaa gaa aca   1337
Leu Glu Ala Met Gly Leu Asp Ser Ser Ala His Asp Pro Lys Glu Thr
    360                 365                 370

ttc ctc agc gac ttc ctc ctg acc cac agg gtc ttc atg ccc agc gcc   1385
Phe Leu Ser Asp Phe Leu Leu Thr His Arg Val Phe Met Pro Ser Ala
375                 380                 385                 390

caa ctc tgc gct gcc ctt ctg cac cac ttc cat gtg gag cct gcg ggt   1433
Gln Leu Cys Ala Ala Leu Leu His His Phe His Val Glu Pro Ala Gly
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ggc agc gag cag gag cgc agc acc tac gtc tgc aac aag agg cag cag   1481
Gly Ser Glu Gln Glu Arg Ser Thr Tyr Val Cys Asn Lys Arg Gln Gln
            410                 415                 420

atc ttg cgg ctg gtc agc cag tgg gtg gcc ctg tat ggc tcc atg ctc   1529
Ile Leu Arg Leu Val Ser Gln Trp Val Ala Leu Tyr Gly Ser Met Leu
        425                 430                 435

cac act gac cct gtg gcc acc agc ttc ctc cac aaa ctc tca gac ctg   1577
His Thr Asp Pro Val Ala Thr Ser Phe Leu His Lys Leu Ser Asp Leu
    440                 445                 450

gtg ggc agg gac acc cga ctc agc aac ctg ctg agg gag cag tgg cca   1625
Val Gly Arg Asp Thr Arg Leu Ser Asn Leu Leu Arg Glu Gln Trp Pro
455                 460                 465                 470

gag agg cgg cga tgc cac agg ttg gag aat ggc tgt ggg aat gca tct   1673
Glu Arg Arg Arg Cys His Arg Leu Glu Asn Gly Cys Gly Asn Ala Ser
                475                 480                 485

cct cag atg aag gcc cgg aac ttg cct gtt tgg ctc ccc aac cag gac   1721
Pro Gln Met Lys Ala Arg Asn Leu Pro Val Trp Leu Pro Asn Gln Asp
            490                 495                 500

gag ccc ctt cct ggc agc agc tgt gcc atc caa gtt ggg gat aaa gtc   1769
Glu Pro Leu Pro Gly Ser Ser Cys Ala Ile Gln Val Gly Asp Lys Val
        505                 510                 515

ccc tat gac atc tgc cgg cca gac cac tca gtg ttg acc ctg cag ctg   1817
Pro Tyr Asp Ile Cys Arg Pro Asp His Ser Val Leu Thr Leu Gln Leu
    520                 525                 530

cct gtg aca gcc tcc gtg aga gag gtg atg gca gcg ttg gcc cag gag   1865
Pro Val Thr Ala Ser Val Arg Glu Val Met Ala Ala Leu Ala Gln Glu
535                 540                 545                 550

gat ggc tgg acc aag ggg cag gtg ctg gtg aag gtc aat tct gca ggt   1913
Asp Gly Trp Thr Lys Gly Gln Val Leu Val Lys Val Asn Ser Ala Gly
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gat gcc att ggc ctg cag cca gat gcc cgt ggt gtg gcc aca tct ctg   1961
Asp Ala Ile Gly Leu Gln Pro Asp Ala Arg Gly Val Ala Thr Ser Leu
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ggg ctc aat gag cgt ctc ttt gtt gtc aac cca cag gaa gtg cat gag   2009
Gly Leu Asn Glu Arg Leu Phe Val Val Asn Pro Gln Glu Val His Glu
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ctg atc cca cac cct gac cag ctg ggg ccc act gtg ggc tct gct gag   2057
Leu Ile Pro His Pro Asp Gln Leu Gly Pro Thr Val Gly Ser Ala Glu
    600                 605                 610

ggg ctg gac ctg gtg agt gcc aag gac ctg gca ggc cag ctg acg gac   2105
Gly Leu Asp Leu Val Ser Ala Lys Asp Leu Ala Gly Gln Leu Thr Asp
615                 620                 625                 630

cac gac tgg agc ctc ttc aac agt atc cac cag gtg gag ctg atc cac   2153
His Asp Trp Ser Leu Phe Asn Ser Ile His Gln Val Glu Leu Ile His
                635                 640                 645

tat gtg ctg ggc ccc cag cat ctg cgg gat gtc acc acc gcc aac ctg   2201
Tyr Val Leu Gly Pro Gln His Leu Arg Asp Val Thr Thr Ala Asn Leu
            650                 655                 660

gag cgc ttc atg cgc cgc ttc aat gag ctg cag tac tgg gtg gcc acc   2249
Glu Arg Phe Met Arg Arg Phe Asn Glu Leu Gln Tyr Trp Val Ala Thr
        665                 670                 675

gag ctg tgt ctc tgc ccc gtg ccc ggc ccc cgg gcc cag ctg ctc aaa   2297
Glu Leu Cys Leu Cys Pro Val Pro Gly Pro Arg Ala Gln Leu Leu Lys
    680                 685                 690

aag ttc att aag ctg gcg gcc cac ctc aag gag cag aag aat gtc aat   2345
Lys Phe Ile Lys Leu Ala Ala His Leu Lys Glu Gln Lys Asn Val Asn
695                 700                 705                 710

tcc ttc ttt gcc gtc atg ttt ggc ctc agc aac tcg ccc atc agc cgc   2393
Ser Phe Phe Ala Val Met Phe Gly Leu Ser Asn Ser Pro Ile Ser Arg
                715                 720                 725

cta gcc cac acc tgg gag cgg ctg cct cac aaa gtc cgg aag ctg tac   2441
Leu Ala His Thr Trp Glu Arg Leu Pro His Lys Val Arg Lys Leu Tyr
            730                 735                 740

tcc gcc ctc gag agg ctg ctg gat ccc tca tgg aac cac cgg gta tac   2489
Ser Ala Leu Glu Arg Leu Leu Asp Pro Ser Trp Asn His Arg Val Tyr
        745                 750                 755

cga ctg gcc ctc gcc aag ctc tcc cct cct gtc atc ccc ttc atg ccc   2537
Arg Leu Ala Leu Ala Lys Leu Ser Pro Pro Val Ile Pro Phe Met Pro
    760                 765                 770

ctt ctt ctc aaa gac atg acc ttc att cat gag gga aac cac aca cta   2585
Leu Leu Leu Lys Asp Met Thr Phe Ile His Glu Gly Asn His Thr Leu
775                 780                 785                 790

gtg gag aat ctc atc aac ttt gag aag atg aga atg atg gcc aga gcc   2633
Val Glu Asn Leu Ile Asn Phe Glu Lys Met Arg Met Met Ala Arg Ala
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gcg cgg atg ctg cac cac tgc cga agc cac aac cct gtg cct ctc tca   2681
Ala Arg Met Leu His His Cys Arg Ser His Asn Pro Val Pro Leu Ser
            810                 815                 820

cca ctc aga agc cga gtt tcc cac ctc cac gag gac agc cag gtg gcg   2729
Pro Leu Arg Ser Arg Val Ser His Leu His Glu Asp Ser Gln Val Ala
        825                 830                 835

agg att tcc aca tgc tcg gag cag tcc ctg agc acc cgg agt cca gcc   2777
Arg Ile Ser Thr Cys Ser Glu Gln Ser Leu Ser Thr Arg Ser Pro Ala
    840                 845                 850

agc acc tgg gct tat gtc cag cag ctg aag gtc att gac aac cag cgg   2825
Ser Thr Trp Ala Tyr Val Gln Gln Leu Lys Val Ile Asp Asn Gln Arg
855                 860                 865                 870

gaa ctc tcc cgc ctg tcc cga gag ctg gag cca tgaggagggg ctgggactgg 2878
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Glu Leu Ser Arg Leu Ser Arg Glu Leu Glu Pro
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35 40 45

Gln Ala Ser Thr Glu Arg Val Leu Arg Ala Gly Arg Gln Leu His Gln
50 55 60

His Leu Leu Ala Thr Cys Pro Asn Leu Ile Arg Asp Arg Lys Tyr His
65 70 75 80

Leu Arg Leu Tyr Arg Gln Cys Cys Ser Gly Arg Glu Leu Val Asp Gly
85 90 95

Ile Leu Ala Leu Gly Leu Gly Val His Ser Arg Ser Gln Val Val Gly
100 105 110

Ile Cys Gln Val Leu Leu Asp Glu Gly Ala Leu Cys His Val Lys His
115 120 125

Asp Trp Ala Phe Gln Asp Arg Asp Ala Gln Phe Tyr Arg Phe Pro Gly
130 135 140

Pro Glu Pro Glu Pro Val Gly Thr His Glu Met Glu Glu Glu Leu Ala
145 150 155 160

Glu Ala Val Ala Leu Leu Ser Gln Arg Gly Pro Asp Ala Leu Leu Thr
165 170 175

Val Ala Leu Arg Lys Pro Pro Gly Gln Arg Thr Asp Glu Glu Leu Asp
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Leu Ile Phe Glu Glu Leu Leu His Ile Lys Ala Val Ala His Leu Ser
195 200 205

Asn Ser Val Lys Arg Glu Leu Ala Ala Val Leu Leu Phe Glu Pro His
210 215 220

Ser Lys Ala Gly Thr Val Leu Phe Ser Gln Gly Asp Lys Gly Thr Ser
225 230 235 240

Trp Tyr Ile Ile Trp Lys Gly Ser Val Asn Val Val Thr His Gly Lys
245 250 255

Gly Leu Val Thr Thr Leu His Glu Gly Asp Asp Phe Gly Gln Leu Ala
260 265 270

Leu Val Asn Asp Ala Pro Arg Ala Ala Thr Ile Ile Leu Arg Glu Tyr
275 280 285

Asn Cys His Phe Leu Arg Val Asp Lys Gln Asp Phe Asn Arg Ile Ile
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Lys Asp Val Glu Ala Lys Thr Met Arg Leu Glu Glu His Gly Lys Val
305 310 315 320

Val Leu Val Leu Glu Arg Ala Ser Gln Gly Ala Gly Pro Ser Arg Pro
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Pro Thr Pro Gly Arg Asn Arg Tyr Thr Val Met Ser Gly Thr Pro Asp
340 345 350

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His Asp Pro Lys Glu Thr Phe Leu Ser Asp Phe Leu Leu Thr His Arg
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Val Phe Met Pro Ser Ala Gln Leu Cys Ala Ala Leu Leu His His Phe
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His Val Glu Pro Ala Gly Gly Ser Glu Gln Glu Arg Ser Thr Tyr Val
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Cys Asn Lys Arg Gln Gln Ile Leu Arg Leu Val Ser Gln Trp Val Ala
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Leu Tyr Gly Ser Met Leu His Thr Asp Pro Val Ala Thr Ser Phe Leu
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His Lys Leu Ser Asp Leu Val Gly Arg Asp Thr Arg Leu Ser Asn Leu
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Leu Arg Glu Gln Trp Pro Glu Arg Arg Arg Cys His Arg Leu Glu Asn
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Gly Cys Gly Asn Ala Ser Pro Gln Met Lys Ala Arg Asn Leu Pro Val
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Trp Leu Pro Asn Gln Asp Glu Pro Leu Pro Gly Ser Ser Cys Ala Ile
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Gln Val Gly Asp Lys Val Pro Tyr Asp Ile Cys Arg Pro Asp His Ser
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Val Leu Thr Leu Gln Leu Pro Val Thr Ala Ser Val Arg Glu Val Met
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Ala Ala Leu Ala Gln Glu Asp Gly Trp Thr Lys Gly Gln Val Leu Val
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Lys Val Asn Ser Ala Gly Asp Ala Ile Gly Leu Gln Pro Asp Ala Arg
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Gly Val Ala Thr Ser Leu Gly Leu Asn Glu Arg Leu Phe Val Val Asn
580 585 590

Pro Gln Glu Val His Glu Leu Ile Pro His Pro Asp Gln Leu Gly Pro
595 600 605

Thr Val Gly Ser Ala Glu Gly Leu Asp Leu Val Ser Ala Lys Asp Leu
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Ala Gly Gln Leu Thr Asp His Asp Trp Ser Leu Phe Asn Ser Ile His
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Gln Val Glu Leu Ile His Tyr Val Leu Gly Pro Gln His Leu Arg Asp
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Val Thr Thr Ala Asn Leu Glu Arg Phe Met Arg Arg Phe Asn Glu Leu
660 665 670

Gln Tyr Trp Val Ala Thr Glu Leu Cys Leu Cys Pro Val Pro Gly Pro
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Arg Ala Gln Leu Leu Lys Lys Phe Ile Lys Leu Ala Ala His Leu Lys
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Glu Gln Lys Asn Val Asn Ser Phe Phe Ala Val Met Phe Gly Leu Ser
705 710 715 720

Asn Ser Pro Ile Ser Arg Leu Ala His Thr Trp Glu Arg Leu Pro His
725 730 735

Lys Val Arg Lys Leu Tyr Ser Ala Leu Glu Arg Leu Leu Asp Pro Ser
740 745 750

Trp Asn His Arg Val Tyr Arg Leu Ala Leu Ala Lys Leu Ser Pro Pro
755 760 765

Val Ile Pro Phe Met Pro Leu Leu Leu Lys Asp Met Thr Phe Ile His
770 775 780

Glu Gly Asn His Thr Leu Val Glu Asn Leu Ile Asn Phe Glu Lys Met
785 790 795 800

Arg Met Met Ala Arg Ala Ala Arg Met Leu His His Cys Arg Ser His
805 810 815

Asn Pro Val Pro Leu Ser Pro Leu Arg Ser Arg Val Ser His Leu His
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Glu Asp Ser Gln Val Ala Arg Ile Ser Thr Cys Ser Glu Gln Ser Leu
835 840 845

Ser Thr Arg Ser Pro Ala Ser Thr Trp Ala Tyr Val Gln Gln Leu Lys
850 855 860

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ggcaggtggg cctggctgtg gaggatagcc cagctctggg agcaccgcgg gtgggagccc 180

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Met Val Leu Arg Arg Met
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cac cgg ccc cga agc tgc tcc tac cag ctg ctg ctg gag cac cag cat 281
His Arg Pro Arg Ser Cys Ser Tyr Gln Leu Leu Leu Glu His Gln His
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Pro Ser Cys Ile Gln Gly Leu Arg Trp Thr Pro Leu Thr Asn Ser Glu
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Glu Ser Leu Asp Phe Ser Glu Ser Leu Glu Gln Ala Ser Thr Glu Arg
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Val Leu Arg Ala Gly Arg Gln Leu His Gln His Leu Leu Ala Thr Cys
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cca aac ctc atc cga gac cgg aag tac cac ctt agg ctc tat cgg cag   473
Pro Asn Leu Ile Arg Asp Arg Lys Tyr His Leu Arg Leu Tyr Arg Gln
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Cys Cys Ser Gly Arg Glu Leu Val Asp Gly Ile Leu Ala Leu Gly Leu
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gat gaa ggt gcc ctc tgc cat gtg aaa cac gac tgg gcc ttc cag gac   617
Asp Glu Gly Ala Leu Cys His Val Lys His Asp Trp Ala Phe Gln Asp
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cga gat gcc caa ttc tac cgg ttc ccc ggg ccc gag ccc gag ccc gtg   665
Arg Asp Ala Gln Phe Tyr Arg Phe Pro Gly Pro Glu Pro Glu Pro Val
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Ser Gln Arg Gly Pro Asp Ala Leu Leu Thr Val Ala Leu Arg Lys Pro
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cca ggt cag cgc acg gat gaa gag ctg gac ctc atc ttt gag gag ctg   809
Pro Gly Gln Arg Thr Asp Glu Glu Leu Asp Leu Ile Phe Glu Glu Leu
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ctg cac atc aag gct gtg gcc cac ctc tcc aac tcg gtg aag cga gaa   857
Leu His Ile Lys Ala Val Ala His Leu Ser Asn Ser Val Lys Arg Glu
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tta gcg gct gtt ctg ctc ttt gaa cca cac agc aag gca ggg acc gtg   905
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gga tct gtc aac gtg gtg acc cat ggc aag ggg ctg gtg acc acc ctg  1001
Gly Ser Val Asn Val Val Thr His Gly Lys Gly Leu Val Thr Thr Leu
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cat gag gga gat gat ttt gga cag ctg gct ctg gtg aat gat gca ccc  1049
His Glu Gly Asp Asp Phe Gly Gln Leu Ala Leu Val Asn Asp Ala Pro
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cgg gca gcc acc atc atc ctg cga gaa tac aac tgt cat ttc ctg cgt  1097
Arg Ala Ala Thr Ile Ile Leu Arg Glu Tyr Asn Cys His Phe Leu Arg
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gtg gac aag cag gac ttc aac cgt atc atc aag gat gtg gag gca aag  1145
Val Asp Lys Gln Asp Phe Asn Arg Ile Ile Lys Asp Val Glu Ala Lys
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acc atg cgg ctg gaa gaa cat ggc aaa gtg gtg ctg gtg ctg gag aga  1193
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gcc tct cag ggc gcc ggc cct tcc cga ccc cca acc cca ggc agg aac  1241
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ttg gag gcc atg gga cta gat tcc agt gct cat gac cca aaa gaa aca   1337
Leu Glu Ala Met Gly Leu Asp Ser Ser Ala His Asp Pro Lys Glu Thr
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ttc ctc agc gac ttc ctc ctg acc cac agg gtc ttc atg ccc agc gcc   1385
Phe Leu Ser Asp Phe Leu Leu Thr His Arg Val Phe Met Pro Ser Ala
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ggc agc gag cag gag cgc agc acc tac gtc tgc aac aag agg cag cag   1481
Gly Ser Glu Gln Glu Arg Ser Thr Tyr Val Cys Asn Lys Arg Gln Gln
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atc ttg cgg ctg gtc agc cag tgg gtg gcc ctg tat ggc tcc atg ctc   1529
Ile Leu Arg Leu Val Ser Gln Trp Val Ala Leu Tyr Gly Ser Met Leu
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cac act gac cct gtg gcc acc agc ttc ctc cag aaa ctc tca gac ctg   1577
His Thr Asp Pro Val Ala Thr Ser Phe Leu Gln Lys Leu Ser Asp Leu
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gtg ggc agg gac acc cga ctc agc aac ctg ctg agg gag cag tgg cca   1625
Val Gly Arg Asp Thr Arg Leu Ser Asn Leu Leu Arg Glu Gln Trp Pro
455                 460                 465                 470

gag agg cgg cga tgc cac agg ttg gag aat ggc tgt ggg aat gca tct   1673
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cct cag atg aag gtg tct gcc tgg ccc cag ttt ctt tcc tct gct cct   1721
Pro Gln Met Lys Val Ser Ala Trp Pro Gln Phe Leu Ser Ser Ala Pro
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Gly Arg Gly Lys Leu Ser Ser His Arg His Thr Leu Gly Ser Leu Ile
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ggt gtt cac ggg gcc ctt gct gca tgt ggt gcc ctg ggc cag gcc gtg   1865
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ggagcacaga aggggaaagg agcaggaggg attccaactc tgccagttag cagctgtgtg 4013
gctttgggca tgttacttaa cctctctgag cctcatttat ttcatccata aaatggaaat 4073
aaaaataata cttttgtcaa aaaaaaaaaa aaaaaa 4109

<210> 14
<211> 556
<212> PRT
<213> Homo sapiens

<400> 14
Met Val Leu Arg Arg Met His Arg Pro Arg Ser Cys Ser Tyr Gln Leu
1 5 10 15

Leu Leu Glu His Gln His Pro Ser Cys Ile Gln Gly Leu Arg Trp Thr
20 25 30

Pro Leu Thr Asn Ser Glu Glu Ser Leu Asp Phe Ser Glu Ser Leu Glu
35 40 45

Gln Ala Ser Thr Glu Arg Val Leu Arg Ala Gly Arg Gln Leu His Gln
50 55 60

His Leu Leu Ala Thr Cys Pro Asn Leu Ile Arg Asp Arg Lys Tyr His
65 70 75 80

Leu Arg Leu Tyr Arg Gln Cys Cys Ser Gly Arg Glu Leu Val Asp Gly
85 90 95

Ile Leu Ala Leu Gly Leu Gly Val His Ser Arg Ser Gln Val Val Gly
100 105 110

Ile Cys Gln Val Leu Leu Asp Glu Gly Ala Leu Cys His Val Lys His
115 120 125

Asp Trp Ala Phe Gln Asp Arg Asp Ala Gln Phe Tyr Arg Phe Pro Gly
130 135 140

Pro Glu Pro Glu Pro Val Gly Thr His Glu Met Glu Glu Glu Leu Ala
145 150 155 160

Glu Ala Val Ala Leu Leu Ser Gln Arg Gly Pro Asp Ala Leu Leu Thr
165 170 175

Val Ala Leu Arg Lys Pro Pro Gly Gln Arg Thr Asp Glu Glu Leu Asp
180 185 190

Leu Ile Phe Glu Glu Leu Leu His Ile Lys Ala Val Ala His Leu Ser
195 200 205

Asn Ser Val Lys Arg Glu Leu Ala Ala Val Leu Leu Phe Glu Pro His
210 215 220

Ser Lys Ala Gly Thr Val Leu Phe Ser Gln Gly Asp Lys Gly Thr Ser
225 230 235 240

Trp Tyr Ile Ile Trp Lys Gly Ser Val Asn Val Val Thr His Gly Lys
245 250 255

Gly Leu Val Thr Thr Leu His Glu Gly Asp Asp Phe Gly Gln Leu Ala
260 265 270

Leu Val Asn Asp Ala Pro Arg Ala Ala Thr Ile Ile Leu Arg Glu Tyr
275 280 285

Asn Cys His Phe Leu Arg Val Asp Lys Gln Asp Phe Asn Arg Ile Ile
290 295 300

Lys Asp Val Glu Ala Lys Thr Met Arg Leu Glu Glu His Gly Lys Val
305 310 315 320

Val Leu Val Leu Glu Arg Ala Ser Gln Gly Ala Gly Pro Ser Arg Pro
325 330 335

Pro Thr Pro Gly Arg Asn Arg Tyr Thr Val Met Ser Gly Thr Pro Asp
340 345 350

Lys Ile Leu Glu Leu Leu Leu Glu Ala Met Gly Leu Asp Ser Ser Ala
355 360 365

His Asp Pro Lys Glu Thr Phe Leu Ser Asp Phe Leu Leu Thr His Arg
370 375 380

Val Phe Met Pro Ser Ala Gln Leu Cys Ala Ala Leu Leu His His Phe
385 390 395 400

His Val Glu Pro Ala Gly Gly Ser Glu Gln Glu Arg Ser Thr Tyr Val
405 410 415

Cys Asn Lys Arg Gln Gln Ile Leu Arg Leu Val Ser Gln Trp Val Ala
420 425 430

Leu Tyr Gly Ser Met Leu His Thr Asp Pro Val Ala Thr Ser Phe Leu
435 440 445

Gln Lys Leu Ser Asp Leu Val Gly Arg Asp Thr Arg Leu Ser Asn Leu
450 455 460

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Leu Arg Glu Gln Trp Pro Glu Arg Arg Arg Cys His Arg Leu Glu Asn
465                 470                 475                 480

Gly Cys Gly Asn Ala Ser Pro Gln Met Lys Val Ser Ala Trp Pro Gln
                485                 490                 495

Phe Leu Ser Ser Ala Pro Pro Gly Leu Gln Ala Pro Pro Ser Pro Pro
            500                 505                 510

Asp Pro Glu Gly Leu Cys Gly Arg Gly Lys Leu Ser Ser His Arg His
        515                 520                 525

Thr Leu Gly Ser Leu Ile Gly Val His Gly Ala Leu Ala Ala Cys Gly
    530                 535                 540

Ala Leu Gly Gln Ala Val Pro Gly Gly Ala Glu Ala
545                 550                 555


<210> 15
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<212> DNA
<213> Rattus norvegicus

<220>
<221> CDS
<222> (3)..(875)
<223> cAMP-GEFII

<400> 15
aa ggt gtg ctc aaa cct aat gat gtt tca gta ttt acg acg ctc acc    47
   Gly Val Leu Lys Pro Asn Asp Val Ser Val Phe Thr Thr Leu Thr
     1               5                  10                  15

att aat gga cgc ctg ttt gcc tgc ccg cga gag caa ttc gac tca ctg   95
Ile Asn Gly Arg Leu Phe Ala Cys Pro Arg Glu Gln Phe Asp Ser Leu
                20                  25                  30

act ccc ttg cca gaa cag gag ggc ccg acc act ggg aca gtg ggg acg   143
Thr Pro Leu Pro Glu Gln Glu Gly Pro Thr Thr Gly Thr Val Gly Thr
            35                  40                  45

ttt gaa ctg atg agc tcg aaa gac ttg gcg tac cag atg aca acg tat   191
Phe Glu Leu Met Ser Ser Lys Asp Leu Ala Tyr Gln Met Thr Thr Tyr
        50                  55                  60

gac tgg gaa ctc ttc aac tgt gtg ctc gag ctg gag cta atc tac cac   239
Asp Trp Glu Leu Phe Asn Cys Val Leu Glu Leu Glu Leu Ile Tyr His
    65                  70                  75

aca ttt gga agg cat aat ttt aaa aag acc aca gca aac ttg gat ttg   287
Thr Phe Gly Arg His Asn Phe Lys Lys Thr Thr Ala Asn Leu Asp Leu
80                  85                  90                  95

ttc ctg agg aga ttt aat gaa att cag ttt tgg gtt gtc act gag atc   335
Phe Leu Arg Arg Phe Asn Glu Ile Gln Phe Trp Val Val Thr Glu Ile
                100                 105                 110

tgc ctt tgt tcc cag ctc agc aag cgt gtt cag ctt ttg aaa aaa tgt   383
Cys Leu Cys Ser Gln Leu Ser Lys Arg Val Gln Leu Leu Lys Lys Cys
            115                 120                 125

atc aag ata gcg gct cac tgc aag gag tac aaa aac ttg aat tcc ttc   431
Ile Lys Ile Ala Ala His Cys Lys Glu Tyr Lys Asn Leu Asn Ser Phe
        130                 135                 140

ttc ggc atc gtc atg ggg ctc agt aac gtg gct gag agc cgc ctg gca   479
Phe Gly Ile Val Met Gly Leu Ser Asn Val Ala Glu Ser Arg Leu Ala
    145                 150                 155

tta aca tgg gag aaa ctg ccg agc aag ttt aag aag ttc tat gcg gag   527
Leu Thr Trp Glu Lys Leu Pro Ser Lys Phe Lys Lys Phe Tyr Ala Glu
160                 165                 170                 175
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ttt gag agc tta atg gat cct tcc aga aat cac aag gcg tac agg ctg   575
Phe Glu Ser Leu Met Asp Pro Ser Arg Asn His Lys Ala Tyr Arg Leu
                180                 185                 190

aca gca gct aaa ctg gag ccc ccc ctc atc cct ttc atg ccc ttg ctt   623
Thr Ala Ala Lys Leu Glu Pro Pro Leu Ile Pro Phe Met Pro Leu Leu
            195                 200                 205

att aaa gat atg aca ttt act cat gag ggg aac aag aca ttc att gac   671
Ile Lys Asp Met Thr Phe Thr His Glu Gly Asn Lys Thr Phe Ile Asp
        210                 215                 220

aat cta gta aac ttt gaa aaa atg cgc atg att gca aat act gcc aga   719
Asn Leu Val Asn Phe Glu Lys Met Arg Met Ile Ala Asn Thr Ala Arg
    225                 230                 235

acg gtg cgc tac tac agg agc cag cca ttc aat ccg gat gct gct caa   767
Thr Val Arg Tyr Tyr Arg Ser Gln Pro Phe Asn Pro Asp Ala Ala Gln
240                 245                 250                 255

gct aat aag aac cat cag gat gtc cgg agt tat gta cgg caa tta aat   815
Ala Asn Lys Asn His Gln Asp Val Arg Ser Tyr Val Arg Gln Leu Asn
                260                 265                 270

gtg att gac aac cag aga act tta tca cag atg tca cac aga tta gag   863
Val Ile Asp Asn Gln Arg Thr Leu Ser Gln Met Ser His Arg Leu Glu
            275                 280                 285

cct cgc agg cca tagacatctg cagtgcccag agtgatgctc cgtctccagt       915
Pro Arg Arg Pro
        290
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ccacaatctt tcaaaagatg ctgtgtatgc tactactgac tgtgttgcta ctagagaatt 975

ccccagaatg agcaagagac acctcctgag agcccctcgg ggccacatcc tgctttccga 1035

ccacacagga gaaggatctg tcttgtctaa cgcggacatg ccgtagctta ggaaccatca 1095

gctgtagtca tcttcttcac ggttggcaca ccaccgcagg ctcacgtgaa ggcataacct 1155

ggcgaggcta caccaggccc ctgacatccc ttcccaggct gttgcagcat gagactgtcc 1215

cgtggatagg tttgacttgg aatcgctgca atgatataat tgaatgattt gtttacttag 1275

caccttattt ggggtctggg ttctggggag ggtgttgacc ataaaagtcc aaattatcca 1335

tcatgttcct ccatcgtcat aatcttacct ctgaaggaat ggaacctcat cacaacacta 1395

tgaaacatta tgactgttca gtctgtaatt tcggaatgat ctatagaata atatgtttac 1455

attgtaactt ttaaaaactt acaaatcagg attacacaca tgagaattcc actaagaaac 1515

accaaggttc ttaatatcgc cagcgttaag atagaaagta acatttcaga agagcacaat 1575

atacacaaaa catttttca aattgaaata ttttcctggg cattaaaaaa cctttccact 1635

acaaatttat tgttactgat gaaaaaaaaa gcatattttc tggacttaaa tgttattaca 1695

aaaatcttaa ttttcagcaa ttgttttgca ctttcagata gattgtaaat aggttatgca 1755

gtcaatggta tagaattatt tatttgctac ataatagaca ttgtgccaaa taattccttt 1815

ttatttattt tattcagtat gaaattttgg agtacatttt ttctgttttc ttaattagac 1875

tacatttaat gtataggaat tgtatgtaca tatctcttct gtaaataaca gccagtatct 1935

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<210> 16
<211> 291
<212> PRT
<213> Rattus norvegicus

<400> 16

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Gly Val Leu Lys Pro Asn Asp Val Ser Val Phe Thr Thr Leu Thr Ile
 1               5                  10                  15

Asn Gly Arg Leu Phe Ala Cys Pro Arg Glu Gln Phe Asp Ser Leu Thr
            20                  25                  30

Pro Leu Pro Glu Gln Glu Gly Pro Thr Thr Gly Thr Val Gly Thr Phe
        35                  40                  45

Glu Leu Met Ser Ser Lys Asp Leu Ala Tyr Gln Met Thr Thr Tyr Asp
    50                  55                  60

Trp Glu Leu Phe Asn Cys Val Leu Glu Leu Glu Leu Ile Tyr His Thr
65                  70                  75                  80

Phe Gly Arg His Asn Phe Lys Lys Thr Thr Ala Asn Leu Asp Leu Phe
                85                  90                  95

Leu Arg Arg Phe Asn Glu Ile Gln Phe Trp Val Val Thr Glu Ile Cys
            100                 105                 110

Leu Cys Ser Gln Leu Ser Lys Arg Val Gln Leu Leu Lys Lys Cys Ile
        115                 120                 125

Lys Ile Ala Ala His Cys Lys Glu Tyr Lys Asn Leu Asn Ser Phe Phe
    130                 135                 140

Gly Ile Val Met Gly Leu Ser Asn Val Ala Glu Ser Arg Leu Ala Leu
145                 150                 155                 160

Thr Trp Glu Lys Leu Pro Ser Lys Phe Lys Lys Phe Tyr Ala Glu Phe
                165                 170                 175

Glu Ser Leu Met Asp Pro Ser Arg Asn His Lys Ala Tyr Arg Leu Thr
            180                 185                 190

Ala Ala Lys Leu Glu Pro Pro Leu Ile Pro Phe Met Pro Leu Leu Ile
        195                 200                 205

Lys Asp Met Thr Phe Thr His Glu Gly Asn Lys Thr Phe Ile Asp Asn
    210                 215                 220

Leu Val Asn Phe Glu Lys Met Arg Met Ile Ala Asn Thr Ala Arg Thr
225                 230                 235                 240

Val Arg Tyr Tyr Arg Ser Gln Pro Phe Asn Pro Asp Ala Ala Gln Ala
                245                 250                 255

Asn Lys Asn His Gln Asp Val Arg Ser Tyr Val Arg Gln Leu Asn Val
            260                 265                 270

Ile Asp Asn Gln Arg Thr Leu Ser Gln Met Ser His Arg Leu Glu Pro
        275                 280                 285

Arg Arg Pro
    290
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<210> 17
<211> 3013
<212> DNA
<213> Homo sapiens

<220>
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<222> (407)..(2953)
<223> cAMP-GEFII

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aatttcaccc aaatctcctt catcagattt gcttatgtgg ttattatgag aatctggaaa 120

agggaataac attatttcgc cagggtgata tggaacaaac tggtatgctg cctggcaggg 180

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tctttggatg ttaaagtatc tgagaccagc agtcaccagg atgctgtgac catctgtacc 240

ctgggaattg ggacggcctt tggagagtcc attctggaca acacaccccg ccatgcaacc 300

atcgttacca gggagagcag tgaactgctc cgcatcgagc agaaggactt caaggcacta 360

tgggagaaat atcgacagta tatggcagga cttctggctc ctccttatg gta tta    415
                                                  Met Val Leu
                                                    1

tgg aaa cgg gct cta aca atg aca gga ttc ctg aca agg aga aca cac   463
Trp Lys Arg Ala Leu Thr Met Thr Gly Phe Leu Thr Arg Arg Thr His
  5                  10                  15

ctc att gaa cct cac gtt cct ctt cgt cct gct aac acc att acc aag   511
Leu Ile Glu Pro His Val Pro Leu Arg Pro Ala Asn Thr Ile Thr Lys
 20                  25                  30                  35

gtc cct tca gag aag atc ctc aga gct gga aaa att tta cga aat gcc   559
Val Pro Ser Glu Lys Ile Leu Arg Ala Gly Lys Ile Leu Arg Asn Ala
                 40                  45                  50

att ctc tct cga gca cct cac atg ata aga gat aga aaa tac cac cta   607
Ile Leu Ser Arg Ala Pro His Met Ile Arg Asp Arg Lys Tyr His Leu
             55                  60                  65

aag aca tac aga caa tgc tgt gtg gga act gaa ctg gtg gac tgg atg   655
Lys Thr Tyr Arg Gln Cys Cys Val Gly Thr Glu Leu Val Asp Trp Met
         70                  75                  80

atc gac gag aca cca tgt gtt cac tcc cgg act caa gct gtt ggc atg   703
Ile Asp Glu Thr Pro Cys Val His Ser Arg Thr Gln Ala Val Gly Met
 85                  90                  95

tgg caa gtc ctg tta gaa gat ggt gtt ctc aac cac gtg gac cag gag   751
Trp Gln Val Leu Leu Glu Asp Gly Val Leu Asn His Val Asp Gln Glu
100                 105                 110                 115

cac cat ttc caa gac ttt tat tta ttc tat cga ttt ctg gat gat gag   799
His His Phe Gln Asp Phe Tyr Leu Phe Tyr Arg Phe Leu Asp Asp Glu
                120                 125                 130

cac gag gat gcc cct ttg cct act gag gag gag aag aag gag tgt gat   847
His Glu Asp Ala Pro Leu Pro Thr Glu Glu Glu Lys Lys Glu Cys Asp
            135                 140                 145

gag gag ctc cag gac acc atg ctg ctg ctg tca cag atg ggc ccc gac   895
Glu Glu Leu Gln Asp Thr Met Leu Leu Leu Ser Gln Met Gly Pro Asp
        150                 155                 160

gcc cac atg agg atg atc ctt cgc aaa cca cct ggc cag agg act gtg   943
Ala His Met Arg Met Ile Leu Arg Lys Pro Pro Gly Gln Arg Thr Val
    165                 170                 175

gat gac cta gag att atc tat gag gag ctt ctt cat att aaa gcc tta   991
Asp Asp Leu Glu Ile Ile Tyr Glu Glu Leu Leu His Ile Lys Ala Leu
180                 185                 190                 195

tcc cat ctt tct acc aca gtg aaa cga gag tta gca ggt gtt ctc att   1039
Ser His Leu Ser Thr Thr Val Lys Arg Glu Leu Ala Gly Val Leu Ile
                200                 205                 210

ttt gag tct cac gcc aaa gga ggg act gtg ttg ttt aac cag ggg gaa   1087
Phe Glu Ser His Ala Lys Gly Gly Thr Val Leu Phe Asn Gln Gly Glu
            215                 220                 225

gaa ggt acc tcc tgg tac att att cta aaa gga tca gtg aat gta gtc   1135
Glu Gly Thr Ser Trp Tyr Ile Ile Leu Lys Gly Ser Val Asn Val Val
        230                 235                 240

att tac ggc aag ggt gtg gtc tgc acc ctg cat gaa gga gat gac ttc   1183
Ile Tyr Gly Lys Gly Val Val Cys Thr Leu His Glu Gly Asp Asp Phe
    245                 250                 255

ggc aag tta gca cta gtg aat gat gcc cca cga gct gcc tct atc gtc   1231
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Gly Lys Leu Ala Leu Val Asn Asp Ala Pro Arg Ala Ala Ser Ile Val
260                 265                 270                 275

tta cga gaa gat aac tgc cat ttc tta aga gta gac aag gag gat ttc   1279
Leu Arg Glu Asp Asn Cys His Phe Leu Arg Val Asp Lys Glu Asp Phe
                280                 285                 290

aac cgg atc cta agg gac gtg gag gcg aat aca gtc aga ctt aaa gaa   1327
Asn Arg Ile Leu Arg Asp Val Glu Ala Asn Thr Val Arg Leu Lys Glu
            295                 300                 305

cat gac caa gat gtc ttg gtg ctg gag aag gtc cca gca ggg aac aga   1375
His Asp Gln Asp Val Leu Val Leu Glu Lys Val Pro Ala Gly Asn Arg
        310                 315                 320

gct tct aat caa gga aac tca cag cct cag caa aag tat act gtg atg   1423
Ala Ser Asn Gln Gly Asn Ser Gln Pro Gln Gln Lys Tyr Thr Val Met
    325                 330                 335

tca gga aca cct gaa aaa att tta gag cat ttt cta gaa aca ata cgc   1471
Ser Gly Thr Pro Glu Lys Ile Leu Glu His Phe Leu Glu Thr Ile Arg
340                 345                 350                 355

ctt gag gca act tta aat gaa gca aca gat tct gtt tta aat gac ttt   1519
Leu Glu Ala Thr Leu Asn Glu Ala Thr Asp Ser Val Leu Asn Asp Phe
                360                 365                 370

att atg atg cac tgt gtt ttt atg cca aat acc cag ctt tgc ccg gca   1567
Ile Met Met His Cys Val Phe Met Pro Asn Thr Gln Leu Cys Pro Ala
            375                 380                 385

ctg gtg gcc cac tac cac gca cag cct tca caa ggt aca gaa cag gag   1615
Leu Val Ala His Tyr His Ala Gln Pro Ser Gln Gly Thr Glu Gln Glu
        390                 395                 400

aaa atg gat tat gcc ctc aac aat aag agg cga gtc atc cgc ctg gtt   1663
Lys Met Asp Tyr Ala Leu Asn Asn Lys Arg Arg Val Ile Arg Leu Val
    405                 410                 415

cta cag tgg gct gcc atg tat gga gac ctc ctg caa gag gat gac gta   1711
Leu Gln Trp Ala Ala Met Tyr Gly Asp Leu Leu Gln Glu Asp Asp Val
420                 425                 430                 435

tct atg gcc ttc ctg gag gag ttt tat gta tct gta tca gat gat gcc   1759
Ser Met Ala Phe Leu Glu Glu Phe Tyr Val Ser Val Ser Asp Asp Ala
                440                 445                 450

cgg atg att gct gcc ctc aag gag caa ctg cca gag ttg gag aag att   1807
Arg Met Ile Ala Ala Leu Lys Glu Gln Leu Pro Glu Leu Glu Lys Ile
            455                 460                 465

gtc aag caa atc tca gaa gat gca aag gca cca caa aag aag cac aag   1855
Val Lys Gln Ile Ser Glu Asp Ala Lys Ala Pro Gln Lys Lys His Lys
        470                 475                 480

gtt ctt ttg caa cag ttc aat acg ggc gat gag aga gcc cag aag cgc   1903
Val Leu Leu Gln Gln Phe Asn Thr Gly Asp Glu Arg Ala Gln Lys Arg
    485                 490                 495

cag cct atc cgc ggc tct gat gaa gtt ctg ttt aag gtc tat tgc atg   1951
Gln Pro Ile Arg Gly Ser Asp Glu Val Leu Phe Lys Val Tyr Cys Met
500                 505                 510                 515

gac cac acc tac aca acc att cgg gtg cca gtg gcc act tcg gtg aag   1999
Asp His Thr Tyr Thr Thr Ile Arg Val Pro Val Ala Thr Ser Val Lys
                520                 525                 530

gaa gtc atc agt gca gtt gcc gac aag ctg ggc tcc ggg gag ggc ctg   2047
Glu Val Ile Ser Ala Val Ala Asp Lys Leu Gly Ser Gly Glu Gly Leu
            535                 540                 545

atc ata gtc aag atg agt tcc gga gga gaa aag gtg gtg ctc aaa cct   2095
Ile Ile Val Lys Met Ser Ser Gly Gly Glu Lys Val Val Leu Lys Pro
        550                 555                 560
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aat gat gtt tca gta ttt acg acg ctc acc att aat gga cgc ctg ttt   2143
Asn Asp Val Ser Val Phe Thr Thr Leu Thr Ile Asn Gly Arg Leu Phe
    565                 570                 575

gct tgc ccg cga gag caa ttc gat tca ctg act ccc tta cca gaa cag   2191
Ala Cys Pro Arg Glu Gln Phe Asp Ser Leu Thr Pro Leu Pro Glu Gln
580                 585                 590                 595

gaa ggc cca act gtt gga aca gtg gga act ttt gaa ctg atg agc tcc   2239
Glu Gly Pro Thr Val Gly Thr Val Gly Thr Phe Glu Leu Met Ser Ser
                600                 605                 610

aaa gat tta gca tac cag atg aca att tat gat tgg gaa ctc ttc aac   2287
Lys Asp Leu Ala Tyr Gln Met Thr Ile Tyr Asp Trp Glu Leu Phe Asn
            615                 620                 625

tgc gtg cat gag ctg gag cta atc tat cac aca ttt gga agg cat aat   2335
Cys Val His Glu Leu Glu Leu Ile Tyr His Thr Phe Gly Arg His Asn
        630                 635                 640

ttt aaa aag acc aca gca aac ttg gat ttg ttc ctg agg aga ttt aat   2383
Phe Lys Lys Thr Thr Ala Asn Leu Asp Leu Phe Leu Arg Arg Phe Asn
    645                 650                 655

gaa att cag ttt tgg gtc gtc act gag atc tgc ctt tgt tct cag ctc   2431
Glu Ile Gln Phe Trp Val Val Thr Glu Ile Cys Leu Cys Ser Gln Leu
660                 665                 670                 675

agc aag cgt gtt cag cta tta aaa aaa ttt att aag ata gca gcc cac   2479
Ser Lys Arg Val Gln Leu Leu Lys Lys Phe Ile Lys Ile Ala Ala His
                680                 685                 690

tgt aag gag tat aaa aat ctg aat tcc ttt ttt gcc atc gtc atg gga   2527
Cys Lys Glu Tyr Lys Asn Leu Asn Ser Phe Phe Ala Ile Val Met Gly
            695                 700                 705

cta agt aac att gct gtg agc cgc ttg gca cta acg tgg gag aaa ctg   2575
Leu Ser Asn Ile Ala Val Ser Arg Leu Ala Leu Thr Trp Glu Lys Leu
        710                 715                 720

cca agc aag ttc aag aag ttc tat gcg gag ttt gaa agt tta atg gac   2623
Pro Ser Lys Phe Lys Lys Phe Tyr Ala Glu Phe Glu Ser Leu Met Asp
    725                 730                 735

cct tca agg aac cac agg gcc tac agg ctg aca gta gct aag ctg gaa   2671
Pro Ser Arg Asn His Arg Ala Tyr Arg Leu Thr Val Ala Lys Leu Glu
740                 745                 750                 755

cct cct ctc atc ccc ttc atg cct ttg ctc att aaa gat atg aca ttt   2719
Pro Pro Leu Ile Pro Phe Met Pro Leu Leu Ile Lys Asp Met Thr Phe
                760                 765                 770

act cat gag ggg aac aag acg ttc att gac aat cta gta aac ttt gaa   2767
Thr His Glu Gly Asn Lys Thr Phe Ile Asp Asn Leu Val Asn Phe Glu
            775                 780                 785

aaa atg cgc atg att gca aat acg gcc aga aca gtg aga tac tac agg   2815
Lys Met Arg Met Ile Ala Asn Thr Ala Arg Thr Val Arg Tyr Tyr Arg
        790                 795                 800

agc caa ccc ttc aat cct gat gca gct caa gct aat aag aac cat cag   2863
Ser Gln Pro Phe Asn Pro Asp Ala Ala Gln Ala Asn Lys Asn His Gln
    805                 810                 815

gat gtc cgg agt tat gta cgg caa tta aat gtg att gac aac cag aga   2911
Asp Val Arg Ser Tyr Val Arg Gln Leu Asn Val Ile Asp Asn Gln Arg
820                 825                 830                 835

act tta tca cag atg tca cac aga tta gag cct cgt cga cca           2953
Thr Leu Ser Gln Met Ser His Arg Leu Glu Pro Arg Arg Pro
                840                 845

tagacatttc aaatgcccaa agcaacagtt tgtctccagt ccacaatttt caaaaatgcc 3013
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<210> 18
<211> 849
<212> PRT
<213> Homo sapiens

<400> 18

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Met Val Leu Trp Lys Arg Ala Leu Thr Met Thr Gly Phe Leu Thr Arg
  1               5                  10                  15

Arg Thr His Leu Ile Glu Pro His Val Pro Leu Arg Pro Ala Asn Thr
            20                  25                  30

Ile Thr Lys Val Pro Ser Glu Lys Ile Leu Arg Ala Gly Lys Ile Leu
        35                  40                  45

Arg Asn Ala Ile Leu Ser Arg Ala Pro His Met Ile Arg Asp Arg Lys
    50                  55                  60

Tyr His Leu Lys Thr Tyr Arg Gln Cys Cys Val Gly Thr Glu Leu Val
65                  70                  75                  80

Asp Trp Met Ile Asp Glu Thr Pro Cys Val His Ser Arg Thr Gln Ala
                85                  90                  95

Val Gly Met Trp Gln Val Leu Leu Glu Asp Gly Val Leu Asn His Val
            100                 105                 110

Asp Gln Glu His His Phe Gln Asp Phe Tyr Leu Phe Tyr Arg Phe Leu
        115                 120                 125

Asp Asp Glu His Glu Asp Ala Pro Leu Pro Thr Glu Glu Glu Lys Lys
    130                 135                 140

Glu Cys Asp Glu Glu Leu Gln Asp Thr Met Leu Leu Leu Ser Gln Met
145                 150                 155                 160

Gly Pro Asp Ala His Met Arg Met Ile Leu Arg Lys Pro Pro Gly Gln
                165                 170                 175

Arg Thr Val Asp Asp Leu Glu Ile Ile Tyr Glu Glu Leu Leu His Ile
            180                 185                 190

Lys Ala Leu Ser His Leu Ser Thr Thr Val Lys Arg Glu Leu Ala Gly
        195                 200                 205

Val Leu Ile Phe Glu Ser His Ala Lys Gly Gly Thr Val Leu Phe Asn
    210                 215                 220

Gln Gly Glu Glu Gly Thr Ser Trp Tyr Ile Ile Leu Lys Gly Ser Val
225                 230                 235                 240

Asn Val Val Ile Tyr Gly Lys Gly Val Val Cys Thr Leu His Glu Gly
                245                 250                 255

Asp Asp Phe Gly Lys Leu Ala Leu Val Asn Asp Ala Pro Arg Ala Ala
            260                 265                 270

Ser Ile Val Leu Arg Glu Asp Asn Cys His Phe Leu Arg Val Asp Lys
        275                 280                 285

Glu Asp Phe Asn Arg Ile Leu Arg Asp Val Glu Ala Asn Thr Val Arg
    290                 295                 300

Leu Lys Glu His Asp Gln Asp Val Leu Val Leu Glu Lys Val Pro Ala
305                 310                 315                 320

Gly Asn Arg Ala Ser Asn Gln Gly Asn Ser Gln Pro Gln Gln Lys Tyr
                325                 330                 335

Thr Val Met Ser Gly Thr Pro Glu Lys Ile Leu Glu His Phe Leu Glu
            340                 345                 350

Thr Ile Arg Leu Glu Ala Thr Leu Asn Glu Ala Thr Asp Ser Val Leu
        355                 360                 365
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Asn Asp Phe Ile Met Met His Cys Val Phe Met Pro Asn Thr Gln Leu
370 375 380

Cys Pro Ala Leu Val Ala His Tyr His Ala Gln Pro Ser Gln Gly Thr
385 390 395 400

Glu Gln Glu Lys Met Asp Tyr Ala Leu Asn Asn Lys Arg Arg Val Ile
405 410 415

Arg Leu Val Leu Gln Trp Ala Ala Met Tyr Gly Asp Leu Leu Gln Glu
420 425 430

Asp Asp Val Ser Met Ala Phe Leu Glu Glu Phe Tyr Val Ser Val Ser
435 440 445

Asp Asp Ala Arg Met Ile Ala Ala Leu Lys Glu Gln Leu Pro Glu Leu
450 455 460

Glu Lys Ile Val Lys Gln Ile Ser Glu Asp Ala Lys Ala Pro Gln Lys
465 470 475 480

Lys His Lys Val Leu Leu Gln Gln Phe Asn Thr Gly Asp Glu Arg Ala
485 490 495

Gln Lys Arg Gln Pro Ile Arg Gly Ser Asp Glu Val Leu Phe Lys Val
500 505 510

Tyr Cys Met Asp His Thr Tyr Thr Thr Ile Arg Val Pro Val Ala Thr
515 520 525

Ser Val Lys Glu Val Ile Ser Ala Val Ala Asp Lys Leu Gly Ser Gly
530 535 540

Glu Gly Leu Ile Ile Val Lys Met Ser Ser Gly Gly Glu Lys Val Val
545 550 555 560

Leu Lys Pro Asn Asp Val Ser Val Phe Thr Thr Leu Thr Ile Asn Gly
565 570 575

Arg Leu Phe Ala Cys Pro Arg Glu Gln Phe Asp Ser Leu Thr Pro Leu
580 585 590

Pro Glu Gln Glu Gly Pro Thr Val Gly Thr Val Gly Thr Phe Glu Leu
595 600 605

Met Ser Ser Lys Asp Leu Ala Tyr Gln Met Thr Ile Tyr Asp Trp Glu
610 615 620

Leu Phe Asn Cys Val His Glu Leu Glu Leu Ile Tyr His Thr Phe Gly
625 630 635 640

Arg His Asn Phe Lys Lys Thr Thr Ala Asn Leu Asp Leu Phe Leu Arg
645 650 655

Arg Phe Asn Glu Ile Gln Phe Trp Val Val Thr Glu Ile Cys Leu Cys
660 665 670

Ser Gln Leu Ser Lys Arg Val Gln Leu Leu Lys Lys Phe Ile Lys Ile
675 680 685

Ala Ala His Cys Lys Glu Tyr Lys Asn Leu Asn Ser Phe Phe Ala Ile
690 695 700

Val Met Gly Leu Ser Asn Ile Ala Val Ser Arg Leu Ala Leu Thr Trp
705 710 715 720

Glu Lys Leu Pro Ser Lys Phe Lys Lys Phe Tyr Ala Glu Phe Glu Ser
725 730 735

Leu Met Asp Pro Ser Arg Asn His Arg Ala Tyr Arg Leu Thr Val Ala
740 745 750

Lys Leu Glu Pro Pro Leu Ile Pro Phe Met Pro Leu Leu Ile Lys Asp
755 760 765

Met Thr Phe Thr His Glu Gly Asn Lys Thr Phe Ile Asp Asn Leu Val
770 775 780

Asn Phe Glu Lys Met Arg Met Ile Ala Asn Thr Ala Arg Thr Val Arg
785 790 795 800

Tyr Tyr Arg Ser Gln Pro Phe Asn Pro Asp Ala Ala Gln Ala Asn Lys
805 810 815

Asn His Gln Asp Val Arg Ser Tyr Val Arg Gln Leu Asn Val Ile Asp
820 825 830

Asn Gln Arg Thr Leu Ser Gln Met Ser His Arg Leu Glu Pro Arg Arg
835 840 845

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, C07K 14/82, 16/32, 14/47, C12Q 1/68, C12N 5/10, A01K 67/027, G01N 33/53, 33/574

A3

(11) International Publication Number: WO 00/24768

(43) International Publication Date: 4 May 2000 (04.05.00)

(21) International Application Number: PCT/US99/24826

(22) International Filing Date: 22 October 1999 (22.10.99)

(30) Priority Data:

60/105,507	23 October 1998 (23.10.98)	US
60/108,685	16 November 1998 (16.11.98)	US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
9 November 2000 (09.11.00)

(54) Title: GENES INTEGRATING SIGNAL TRANSDUCTION PATHWAYS

(57) Abstract

The present invention describes the identification, isolation, sequencing and characterization of two human CalDAG–GEF, and two human cAMP–GEF genes, which are associated with the Ras pathway. Also identified are CalDAG–GEF gene homologues in mice and cAMP–GEF gene homologues in rats. Nucleic acids and proteins comprising or derived from the CalDAG–GEFs and/or cAMP–GEFs are useful in screening and diagnosing certain Ras–associated cancers, in identifying and developing therapeutics for treatment of certain Ras–associated cancers, and in producing cell lines and transgenic animals useful as models of Ras–associated cancers.

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INTERNATIONAL SEARCH REPORT

Internati Application No
PCT/US /24826

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/82 C07K16/32 C07K14/47 C12Q1/68
C12N5/10 A01K67/027 G01N33/53 G01N33/574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K C12Q A01K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EBINU, J.O. ET AL.: "RasGRP, a Ras guanyl nucleotide-releasing protein with calcium- and diacylglycerol-binding motifs." SCIENCE, (1998 MAY 15) 280 (5366) 1082-6, XP000882708 the whole document ---	1
A	GOTOH, T. ET AL.: "Identification of Rap1 as a target for the Crk SH3 domain-binding guanine nucleotide-releasing factor C3G." MOLECULAR AND CELLULAR BIOLOGY, (1995) 15 (12) 6746-53, XP000881340 the whole document ---	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
5 April 2000	3 July 2000 (03.07.00)

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nichogiannopoulou, A

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAIBUCHI, K. ET AL.: "Molecular cloning of the cDNA for stimulatory GDP/GTP exchange protein fo smg p21s (ras p21-like small GTP-binding proteins) and characterization o stimulatory GDP/GTP exchange protein." MOLECULAR AND CELLULAR BIOLOGY, (1991) 11 (5) 2873-80, XP000881341 the whole document	1
X	KEDRA D ET AL: "THE GERMINAL CENTER KINASE GENE AND A NOVEL CDC25-LIKE GENE ARE LOCATED IN THE VICINITY OF THE PYGM GENE ON 11Q13" HUM. GENET., vol. 100, 1 October 1997 (1997-10-01), pages 611-619, XP002069545 page 613, last paragraph -page 615, paragraph FIRST; figure 2 & DATABASE EMBL [Online] AC Y12336, 19 June 1997 (1997-06-19) KEDRA D ET AL: "H. sapiens mRNA for F25B3.3 kinase like protein from C. elegans" Protein with 96.9% identity to SEQ ID No:2 and 100% identity to SEQ ID No:4 the whole document	1,3,5-8, 10, 38-54, 63-69
P,X	WO 98 53061 A (QUEENSLAND INST MED RES; HANCOCK JOHN (AU); SILINS GINTERS (AU)) 26 November 1998 (1998-11-26) MCG7, a hu protein with 100% identity in 609 aa overlap with SEQ ID No:4 claim 5; figure 13B	1,3,5-8, 10, 38-54, 63-69
P,X	KAWASAKI H ET AL: "A Rap guanine nucleotide exchange factor enriched highly in the basal ganglia" PROC. NATL. ACAD. SCI. USA, vol. 95, October 1998 (1998-10), pages 13278-13283, XP000882748 the whole document	1,3,5-8, 10, 38-54, 63-69

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INTERNATIONAL SEARCH REPORT

Intern[illegible]al application No.
PCT/US 99/24826

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims76-81, 86-109 and 127 -in as far as they concern in vivo methods- are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1,3-8,10,12-19,28-32,38-70,72,73-75,76,78-82,84,86,88-98,110-112,116,118,119,121-129 (all partially)

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1, 3-8, 10, 12-19, 28-32, 38-70, 72, 73-75, 76, 78-82, 84, 86, 88-98, 110-112, 116, 118, 119, 121-129 (all partially)

Claims relating to CalDAG-GEFI proteins and nucleic acids encoding them, mutants, variants, species orthologs and homologues, functional domains and antigenic determinants thereof. Methods for identifying variants or homologues of such proteins. Recombinant expression vectors, host cells and animal models for cancer expressing such recombinant modified proteins. Methods for producing said proteins and pure preparations of such. Antibodies selectively binding to such proteins, methods and cell lines for producing them. Methods for identifying compounds that modulate the expression of such proteins, and for identifying compounds that can selectively bind to them. Diagnostic methods for detecting mutations and pharmaceutical preparations comprising pure protein, expression vectors encoding the protein or antisense sequences. Pharmaceutical preparations comprising antibodies or antigenic determinants and methods of treatment.

2. Claims: 1, 3-8, 10, 12-19, 28-32, 38-70, 72, 73-75, 76, 78-82, 84, 86, 88-98, 110-112, 116, 118, 119, 121-129 (all partially)

As in subject 1, the proteins being CalDAG-GEFII proteins.

3. Claims: 2, 4-7, 9, 11, 20-27, 33-69, 71-75, 77-81, 83, 85, 87, 99-109, 113-115, 117, 118, 120-126, 130 (all partially)

As in subject 1, the proteins being cAMP-GEFI proteins.

4. Claims: 2, 4-7, 9, 11, 20-27, 33-69, 71-75, 77-81, 83, 85, 87, 99-109, 113-115, 117, 118, 120-126, 130 (all partially)

As in subject 1, the proteins being cAMP-GEFII proteins.

INTERNATIONAL SEARCH REPORT

Internati[redacted]plication No
PCT/US 99/24826

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9853061 A	26-11-1998	AU 7512998 A	11-12-1998